

Vitamin B₁₂



MERCK SERVICE BULLETIN

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Chemical Division

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Foreword

This *Service Bulletin*, another in the Merck series presents comprehensive, up-to-date information on vitamin B₁₂. Its primary purpose is to aid the pharmaceutical manufacturer, and it is hoped that much of the material will prove equally helpful to others who are interested in the subject. Information essential to the development of new product forms and to the improvement of existing ones is included, as well as basic facts on the nature, physiologic role and clinical uses of the vitamin.

Hence in this volume on Vitamin B₁₂ you will find—

Chemical, Pharmaceutical, and Analytical Information

including data on the chemistry and physical properties of vitamin B₁₂

pharmaceutical applications, with technical details concerning injectable solutions containing vitamin B₁₂, tablets of vitamin B₁₂, alone or with other B vitamins, and multivitamins, capsules, oral liquids, plus a discussion of compatibilities

assay methods chemical, biological, and microbiological

Nutritional, Metabolic, Physiologic, Pharmacologic, and Clinical Information

comprehensive discussions of the roles of vitamin B₁₂ in human nutrition and metabolism are presented, together with data on absorption and excretion

clinical uses in anemias, neurologic disorders, dermatoses, and other conditions are detailed, together with clinical indications for dietary supplementation

Selected Annotated Bibliography—of more than 300 abstracts of selected material appearing in the medicinal and related scientific literature since July 1953

To assist manufacturers still further Merck Technical Service is always ready with even more recent information gained by Merck vitamin research, pharmaceutical experience and analyses of current publications—for the solution of your specific problems

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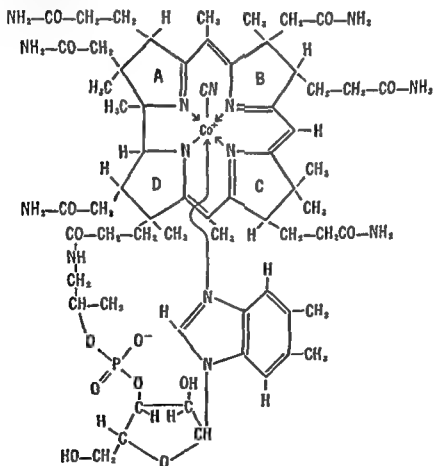
Part 1

Chemical, Pharmaceutical
and
Analytical Information

CHEMISTRY AND PHYSICAL PROPERTIES

Vitamin B₁₂, also known as cyanocobalamin, is a complex coordination compound containing trivalent cobalt with a coordination number of six. Cyanocobalamin has a net charge of zero. The cyano group contributes one negative charge and satisfies one coordinate position. The cobalt is coordinated to one other negatively charged group and to four groups that carry no local charge. All of these coordinating atoms are nitrogen and all five of these groups are linked together into a single moiety. The remaining positive charge in the cobalt coordination sphere is balanced by the negatively charged phosphate portion of the molecule.

Extensive structural and degradation studies of cyanocobalamin have shown it to have an empirical composition corresponding to the formula $C_{63}H_{88}N_{14}O_{14}P$ Co and a molecular weight of 1355. By a combination of chemical and X-ray crystallographic studies the molec



ular structure has been established, with reasonable certainty, and the formula on the preceding page has been suggested ⁸

Vitamin B₁₂ has been specifically designated cyanocobalamin, the name cobalamin thus referring to all of the molecule except the cyano group. A new system of nomenclature for the analogs and degradation products of vitamin B₁₂ has been proposed ⁹. The fundamental ring system consisting of the four pyrrole nuclei joined in a macro ring with the three bridge carbon atoms and six conjugated double bonds, but without cobalt or side chains, is called "corrin". Cyanocobalamin and its degradation products can be named as corrin derivatives but for convenience other names have been assigned. For example the cobalt-containing heptacarboxylic acid resulting from hydrolysis of all of the amide groups present in vitamin B₁₂, without the cyano group and the nucleotide, is designated "corphinic acid". The corresponding hexacarboxylic acid which still retains the 2-hydroxy propionamide group on the appropriate side chain is called "corphinic acid" and the hexacarboxylic acid having the ribofuranosidophosphorylpropionamide side chain is called "cobamic acid". Thus cobamide, the hexamide of cobamic acid, would be derived from vitamin B₁₂ by removal of the cyano and the 5,6-dimethylbenzimidazole groups. By this proposed system of nomenclature cyanocobalamin may be called "cyano 5,6-dimethylbenzimidazolyl cobamide" or "5,6-dimethylbenzimidazole cyanocobamide".

Cyanocobalamin as a whole, is a neutral molecule. The cyano group attached to the cobalt atom can be replaced by other ions or groups with strong coordinating tendencies to yield other cobalamins¹⁰ such as hydroxocobalamin, chlorocobalamin, nitrocobalamin, and thiocyanatocobalamin. Hydroxocobalamin combines with hydrogen ion to form positively charged aquocobalamin which can form salts with anions less strongly coordinated than water. All these cobalamins are readily converted to cyanocobalamin by treatment with cyanide ion. In alkaline solutions containing an excess of cyanide ion, cyanocobalamin takes up a second cyanide group to form dicyanocobalamin, the so-called "purple complex," which is unstable and exists only in solution in an excess of the cyanide ion.

In glacial acetic acid solution, vitamin B₁₂ reacts with perchloric acid to form an orange colored perchlorate. The compound is of structural interest insofar as its formation confirms the presence of at least six weakly basic groups in the vitamin molecule ¹¹

ture The anhydrous compound, however, is very hygroscopic, and when exposed to moist air may absorb about 12 per cent of water Although vitamin B₁₂ is slowly decomposed by ultraviolet or visible light, the crystalline solid and its aqueous solutions show no significant decomposition during exposure at room temperature to normal indoor illumination or indirect sunlight for the time normally required for pharmaceutical processing Prolonged exposure to strong light, however, should be avoided

Crystalline vitamin B₁₂ is stable at normal temperatures Aqueous solutions at pH 4.0 to 7.0 show no decomposition during extended storage at 25°C For optimum stability at elevated temperatures, solutions should be adjusted to pH 4.0 to 4.5 Aqueous solutions in this pH range may be autoclaved for 20 minutes at 120°C without significant decomposition

Cobalamin Concentrate N F (Vitamin B₁₂ Activity Concentrate) is a partially purified product, resulting from the growth of a cobalamin producing microorganism, which contains harmless diluents or stabilizing agents It occurs as pink to brown powder or granules which may be hygroscopic The chemical and physical properties vary depending upon the specific composition The potency is stated in terms of the number of micrograms of cobalamin in each gram of cobalamin concentrate The cobalamin content is determined by the Cobalamin Assay described in the Second Interim Revision Announcement, National Formulary X²⁵ The identity tests and other requirements for Cobalamin Concentrate N F are described on page 167 of The National Formulary, Tenth Edition

Cobalamin Concentrates (Vitamin B₁₂ Solids) N F, adjusted to a uniform potency of either 500 or 1,000 mg of cobalamin (vitamin B₁₂ activity) per Kg with anhydrous dibasic calcium phosphate, are supplied, for pharmaceutical manufacturing in screw capped bottles or fiber drums containing from 100 mg to 10 Gm of cobalamin

Cobalamin Concentrates (Vitamin B₁₂ Solids) N F, adjusted to a uniform potency of either 1,000 mg or 3,000 mg of cobalamin (vitamin B₁₂ activity) per Kg with a water soluble carrier, mannitol, are supplied, for pharmaceutical manufacturing, in screw-capped bottles or fiber drums containing from 100 mg to 10 Gm of cobalamin

A sterile aqueous solution of Cobalamin Concentrate N F, adjusted to a minimum potency of 5,000 micrograms of vitamin B₁₂ activity per cc, is supplied, for manufacture of parenteral solutions,

in 200, 1,000 and 5,000 cc bottles containing 1, 5 and 25 Gm, respectively, of cobalamin. This solution also contains 1.5 per cent of benzyl alcohol as a preservative.

In all of the Cobalamin Concentrates N F supplied by Merck, the labeled cobalamin content is present entirely as cyanocobalamin in noncrystalline form.

PHARMACEUTICAL APPLICATIONS

Some of the pharmaceutical properties of crystalline vitamin B₁₂ were described soon after it became available for therapeutic and nutritional use²⁸⁻³⁰. Experience in the manufacture of a wide variety of pharmaceutical products, as well as critical studies employing accelerated stability tests and long term storage tests, have shown that many stable products containing crystalline vitamin B₁₂ may be prepared by conventional manufacturing procedures. Crystalline vitamin B₁₂ has been shown to be compatible with a wide variety of therapeutic and nutritional substances³¹. Those few substances which are chemically incompatible with vitamin B₁₂ in aqueous solutions may be incorporated into many dry formulations by special procedures which minimize their adverse effect on the stability of the vitamin.

The procedures to be described for the preparation of various dosage forms of vitamin B₁₂ have been used in the experimental production of pharmaceutical formulations. Manufacturers adapting these methods to their own operations should first prepare trial lots in order that a finished product meeting their specific requirements may be assured.

INJECTABLE SOLUTIONS CONTAINING VITAMIN B₁₂

Aqueous solutions of vitamin B₁₂ for parenteral administration are usually prepared in a saline vehicle containing a suitable preservative. Vitamin B₁₂ in solution is sensitive to ultraviolet or visible light and during all stages of processing should be protected from direct sunlight and bright artificial light. Exposure to bright light should also be minimized during subdividing, inspection and packaging. Since vitamin B₁₂ is a growth stimulant for many microorganisms, the solutions should be sterilized as soon as possible.

Only glass, glass lined, or enameled equipment should be used in processing the saline solutions. Corrosion of stainless steel equipment by saline solutions may introduce metallic contaminants. Stainless

steel needles have been found suitable for use in filling equipment, however, because of the relatively short period of contact. A surgical grade of rubber tubing should be used, if needed, for connections in the filling equipment.

Crystalline vitamin B₁₂ (Cyanocobalamin U S P) or a suitable solution of cobalamin concentrate may be used for the manufacture of parenteral solutions.

Single-Dose All-Glass Ampuls of Vitamin B₁₂

Solutions for subdivision into single dose ampuls may be prepared with water for injection or with an isotonic solution of sodium chloride in pyrogen-free distilled water. The pH of the vehicle should be adjusted to between 4.0 and 4.5 before the vitamin B₁₂ is added. The required amount of crystalline vitamin B₁₂, calculated on the anhydrous basis (including 5 per cent overage), is dissolved in the aqueous vehicle. To prevent bacterial growth and subsequent loss of potency during subdivision, the solution is immediately sterilized by filtration through a microporous porcelain candle. The candle should be autoclaved and aseptically rinsed with water before use. The solution is collected in sterile bulk containers and aseptically subdivided into previously washed and sterilized ampuls of Type I glass. The ampuls are sealed and immediately sterilized by autoclaving for 20 minutes at 120°C.

Multiple-Dose Vials of Vitamin B₁₂

Injectable solutions containing crystalline vitamin B₁₂ may be prepared with water for injection or with an isotonic solution of sodium chloride in pyrogen free distilled water. The vehicle should contain a suitable bacteriostatic agent and the pH should be adjusted to 4.0 to 4.5 before the vitamin B₁₂ is added. The vehicle may be clarified, if necessary, by filtering through a fritted glass filter of medium porosity. The desired weight of crystalline vitamin B₁₂, calculated on the anhydrous basis (including 5 per cent overage), is dissolved in the aqueous vehicle.

The solution is immediately sterilized by filtration through a microporous porcelain candle which has been autoclaved and then aseptically rinsed with water. Heat sterilization of the solution is not recommended when a bacteriostatic agent is used. The filtered solution is collected in suitable sterile bulk containers, and then subdivided into

previously washed and sterilized vials of Type I glass. The vials are immediately sealed by affixing sterile stoppers which have been previously tested for compatibility with vitamin B₁₂ solutions.

The stoppers are washed in a suitable detergent solution, rinsed with distilled water until free of detergent, then immersed in distilled water and autoclaved at 120°C for 20 minutes. After the water is poured off, the stoppers are rinsed with distilled water. Sufficient distilled water is added to cover the stoppers which are again autoclaved at 120°C for 20 minutes. After rinsing with distilled water, the stoppers are dried and sterilized.

Vitamin B₁₂ with Other B Vitamins

Injectable solutions containing crystalline vitamin B₁₂ and other B vitamins may be prepared using an isotonic solution of sodium chloride in pyrogen free distilled water as the vehicle.

About 90 per cent of the required amount of isotonic sodium chloride solution is heated to 95°C. The niacinamide (including 5 per cent overage) is dissolved in the saline solution, and riboflavin (including 5 per cent overage) is added and stirred until solution is complete. The solution is then allowed to cool to room temperature.

Thiamine hydrochloride, ampul grade (including 20 per cent overage), pyridoxine hydrochloride (including 10 per cent overage) and calcium pantothenate dextrorotatory (including 50 per cent overage) are dissolved in the cooled solution. The solution is immediately adjusted to pH 4.0 to 4.5 and a suitable preservative is added.

The required amount of crystalline vitamin B₁₂, calculated on the anhydrous basis, is dissolved in the solution and sufficient quantity of the isotonic sodium chloride vehicle added to make the desired total volume. An overage of 10 per cent of crystalline vitamin B₁₂ has been found adequate in solutions containing up to 10 mg per cc of thiamine hydrochloride. When higher concentrations of thiamine are employed, adequate testing must be done to determine the necessary overage.

The solution is sterilized by filtration, using a microporous porcelain candle which has been autoclaved and then aseptically rinsed with water.

The sterile solution is collected in suitable sterile bulk containers. It is then subdivided under aseptic conditions either into previously washed and sterilized glass ampuls which are immediately flame

sealed, or into previously washed and sterilized vials which are immediately sealed with sterile stoppers. Stoppers of a quality suitable for use with such solutions should be washed and prepared as described above.

Vitamin B₁₂ and Other B Vitamins with Liver

Injectable solutions containing crystalline vitamin B₁₂ and other B vitamins with liver may be prepared by the method described for injectable solutions containing vitamin B₁₂ with other B vitamins. The required amount of Liver Injection Crude U.S.P. is added before the pH of the solution containing the other B vitamins is adjusted. The solution is then adjusted to pH 4.0 to 4.5 and a suitable bacteriostatic agent added. The required amount of crystalline vitamin B₁₂, calculated on the anhydrous basis (including 10 per cent overage), is dissolved in this solution and sufficient quantity of the isotonic sodium chloride vehicle added to make the desired total volume.

The solution is sterilized by filtration, using a microporous porcelain candle which has been autoclaved and then aseptically rinsed with water. The sterile solution is collected in suitable sterile bulk containers, and stored at about 5°C for one week to permit coalescence of any insoluble material.

If necessary, the solution is again aseptically filtered as described above and the clear solution is subdivided under aseptic conditions into previously washed and sterilized vials.

TABLETS

Oral tablets containing vitamin B₁₂ alone or in combination with the other B vitamins may be prepared by conventional procedures as described.

Tablets of Vitamin B₁₂

Tablets containing vitamin B₁₂ may be prepared by a wet granulation procedure using as the diluent a powdered mixture of 7 parts of mannitol and 1 part of dibasic calcium phosphate.

The required amounts of a suitable trituration containing crystalline vitamin B₁₂ or cobalamin concentrate (including 10 per cent overage) and the diluent are thoroughly mixed and sifted through a 40-mesh stainless steel screen. The powder is moistened with a freshly prepared 10 per cent cornstarch paste. The wet mass is forced through

an 8 mesh stainless steel screen and dried at 40°C. The dried granules are forced through a 14 mesh stainless steel screen and then lubricated with a mixture of magnesium stearate and cornstarch. The granules are then compressed into tablets.

Soluble, flavored tablets of crystalline vitamin B₁₂ which are easily crushed, yet hard enough to withstand careful handling, may be manufactured by the following method. A lactose granule is prepared by moistening 94 parts of lactose with distilled water containing a suitable red dye. The damp mass is passed through a 12 mesh stainless steel screen and dried first at room temperature for approximately 16 hours, then at 40°C for 1 hour. The dried granules are forced through a 16-mesh screen and the fines removed through a 40-mesh screen. The required amount of 0.1 per cent Trituration of Crystalline Vitamin B₁₂ with Mannitol is mixed with sufficient mannitol to provide 5 parts of the total weight of the compression mixture. Suitable quantities of sodium saccharin and flavor are added and the mixture is thoroughly blended with the fines from the lactose granule. This powdered mixture and 1 part of magnesium stearate are added to the dried lactose granules and, after thorough mixing, compressed into tablets of suitable hardness.

Tablets of Vitamin B₁₂ with Other B Vitamins

Oral tablets of the B vitamins with vitamin B₁₂ may be prepared by mixing the required amounts of a prepared trituration containing crystalline vitamin B₁₂ or cobalamin concentrate, riboflavin, pyridoxine hydrochloride and niacinamide, including a 10 per cent overage of each vitamin. A diluent, consisting of a mixture of 4 parts of mannitol and 1 part of cornstarch, is added during continuous blending. The weight of the diluent should be at least five times that of the vitamin mixture.

After sifting the mixture through a 40 mesh stainless steel screen, the powder is moistened with a warm 10 per cent solution of gelatin and the wet mass pressed through an 8 mesh stainless steel screen. The resulting granules are dried at 40°C.

The dried granules are forced through a 14 mesh stainless steel screen. The fines are removed through a 40 mesh stainless steel screen and divided into three portions. The required amount of thiamine mononitrate (including 10 per cent overage) is mixed with one portion of the fines and sifted onto the granules through the 40 mesh screen.

The required amount of calcium pantothenate dextrorotatory (including 50 per cent overage) is mixed with a second portion of the fines and sifted onto the granules through the 40-mesh screen. The third portion of fines is thoroughly mixed with suitable lubricants and sifted onto the granules through a 40 mesh screen. The lubricated granules are then mixed and compressed into tablets.

Tablets of Vitamin B₁₂ and Intrinsic Factor

Tablets containing vitamin B₁₂ and intrinsic factor concentrate may be prepared by a "slugging" (dry compression) procedure. The required amount of Vitamin B₁₂ with Intrinsic Factor Concentrate U S P is mixed with suitable diluents such as lactose or dicalcium phosphate and a lubricant such as magnesium stearate. The mixture is compressed into slugs which are reduced to a 12 mesh granule, and then recompressed into tablets of the desired size.

Multivitamin Tablets

Oral multivitamin tablets, such as the Decavitamin Tablet described in U S P XV, usually include vitamins A and D, ascorbic acid, *d* calcium pantothenate, crystalline vitamin B₁, folic acid, niacinamide, pyridoxine hydrochloride, riboflavin and thiamine hydrochloride or mononitrate. The quantities of the components may be varied to prepare tablets containing the desired maintenance or therapeutic levels.

In the preparation of such tablets the required amount of a suitable trituration containing crystalline vitamin B₁₂ is mixed with the required amounts of riboflavin, niacinamide and pyridoxine hydrochloride, including 10 per cent overage of each vitamin. A separate granulation of this mixture is prepared by conventional procedures using suitable granulating solutions such as 2 per cent ethyl cellulose in 95 per cent alcohol or 10 per cent zein in warm 95 per cent alcohol.

The required amounts of granular vitamins A and D (including 10 per cent overage), powdered thiamine mononitrate (including 10 per cent overage), powdered *d* calcium pantothenate (including 50 per cent overage), and ascorbic acid, fine or medium crystals (including 10 per cent overage), are added to the dried granulation together with suitable lubricants and thoroughly blended. The mixture is then compressed into tablets and a sugar coating applied by conventional methods.

may be advisable in dry filled capsules containing reactive mixtures which are influenced by moisture

The incorporation of vitamin B₁₂ alone or in combination with vitamin mixtures and other substances with which it is compatible into hard gelatin capsules is accomplished by mixing the components with suitable dry extenders such as cornstarch, mannitol, or dicalcium phosphate. The methods used in preparing uniform mixtures of dry powders for tablets are applicable to capsule manufacture. A small amount of magnesium stearate may be added as a lubricant.

Methods for incorporating vitamin B₁₂ and other vitamins into soft gelatin capsules have been developed by manufacturers possessing the highly specialized equipment required for this product form. The desired combination of vitamins is usually mixed with a suitable base to form a smooth paste which is then encapsulated in soft gelatin on automatic or semiautomatic machines.

ORAL LIQUIDS

Oral liquid vitamin formulations are usually prepared in vehicles containing sucrose, sorbitol, glycerin, propylene glycol, or combinations of these, with varying amounts of water. Such vehicles with low water content have been found suitable for use in liquid multivitamin preparations containing crystalline vitamin B₁₂.

A typical oral liquid formulation containing vitamin B₁₂ with other B vitamins and hematinic substances may be prepared in a vehicle containing 90 parts (weight/volume) of an invert sugar concentrate consisting of 60 per cent invert sugar and 40 per cent sucrose.

The required amounts of ferrous sulfate and Liver Extract U S P and sufficient citric acid to adjust the final product to pH 3.5 to 4.0 are dissolved, with stirring, in half of the volume of distilled water required to provide a final volume of 100 parts. The solution is kept at room temperature for 48 hours and then clarified by filtration through a suitable cloth filter.

The required amounts of niacinamide, riboflavin, and a suitable preservative are dissolved, with the aid of heat, in the total quantity of invert sugar concentrate. This solution is cooled to about 35°C, and the aqueous liver and iron solution is then added.

The required amounts of thiamine hydrochloride, pyridoxine hydrochloride, choline chloride, and crystalline vitamin B₁₂ are dissolved in the remainder of the water. Suitable flavoring and coloring

agents are added and this solution is mixed with the invert sugar solution containing the liver and iron

The final product is clarified by filtration and subdivided into bottles

COMPATIBILITY IN PHARMACEUTICAL PRODUCTS

Cyanocobalamin is more stable than any of the other cobalamins in pharmaceutical formulations even in the presence of reducing substances

In all types of dry pharmaceutical formulations crystalline vitamin B₁₂ is usually stable for prolonged periods. The purity of the other components, however, becomes important because the concentrations of vitamin B₁₂ employed are usually relatively minute. In liquid formulations, trace contaminants, the concentration of the individual components and storage conditions have a marked influence on the stability of vitamin B₁₂. Only a few substances are known to have an adverse effect on vitamin B₁₂ stability. Ascorbic acid causes the most serious difficulty particularly in liquid formulations. Other reducing substances such as ferrous salts and aldehydes may also cause decomposition of vitamin B₁₂ in some types of pharmaceutical products. Specially prepared powdered products containing crystalline vitamin B₁₂ in gelatin have been used successfully in dry formulations containing reducing substances such as ascorbic acid and ferrous sulfate.

The most important stability problems in pharmaceutical preparations are concerned with the combination of vitamin B₁₂ with ascorbic acid in liquid oral products and its combination with thiamine hydrochloride and niacinamide in B complex parenteral solutions. Cyanocobalamin, however, was found to be the most stable of a series of cobalamin analogs tested in ascorbic acid solutions in 1 molar acetate buffer at pH 4.0. Vitamin B₁₂ activity concentrates in which all of the activity is present as cyanocobalamin are more stable in such solutions than concentrates containing other cobalamin analogs.¹⁶

The adverse effect of ascorbic acid on vitamin B₁₂ stability in oral liquid multivitamin preparations can be reduced by use of vehicles of low water content. Sorbitol, propylene glycol or glycerin solutions containing varying amounts of water have been used. Vitamin B₁₂ in combination with ascorbic acid in a vehicle composed of equal parts of propylene glycol and glycerin has been reported to be stable at room temperature for prolonged periods.¹⁷

The stability of vitamin B₁ in solutions of other B vitamins may vary, depending upon the concentration of thiamine hydrochloride and niacinamide present, the storage temperatures, and the pH of the solution. At room temperature, vitamin B₁ is stable for prolonged periods in solutions containing as much as 10 mg per cc of thiamine hydrochloride and an equal quantity of niacinamide. At levels of 25 to 100 mg per cc of thiamine hydrochloride and niacinamide, vitamin B₁ may decompose during normal storage periods even at room temperature.²⁰ Since the rate of decomposition of vitamin B₁₂ in such combinations appears to be directly related to thiamine decomposition, parenteral solutions should be formulated to provide optimum thiamine stability. The presence of even small amounts of thiamine decomposition products, or more specifically, those derived from the thiazole moiety, causes rapid decomposition of vitamin B₁₂ at elevated temperatures.²¹ This effect is more pronounced in the presence of niacinamide, although niacinamide itself has been shown to have no adverse effect on vitamin B₁₂ stability in solutions held at 120°C for 2 hours.²²

Under normal storage conditions, no serious stability problems have been encountered with vitamin B₁₂ in combination with the common sugars in dry mixtures, dilute solutions or syrups. In test solutions heated to 120°C, however, significant decomposition of vitamin B₁₂ has occurred in solutions containing sucrose. Decomposition to a lesser degree has occurred in similar solutions containing dextrose and lactose, but solutions of vitamin B₁₂ containing mannitol showed no decomposition after heating to 120°C for several hours.

Although talc cannot be considered incompatible with vitamin B₁₂, assay difficulties may result from its use as a filter aid or tablet component because of its ability to adsorb the vitamin.

A rapid method for determining chemical compatibility in aqueous solution consists of heating a buffered solution of crystalline vitamin B₁ and the material to be tested at 100°C for 4 hours. If the assays of this solution and a control solution containing only the buffer and the vitamin B₁₂ differ by less than 10 per cent, the substance under test can be presumed to be compatible with vitamin B₁₂. A 1 M sodium acetate—acetic acid buffer at pH 4 has been found satisfactory for this test. Decomposition of vitamin B₁₂ in the test solutions at 100°C does not necessarily imply that decomposition will occur in the finished product under normal storage conditions.

ASSAY METHODS

Biologically, vitamin B₁₂ is one of the most potent substances ever discovered. Before vitamin B₁₂ was discovered and characterized as a vitamin, the activity of liver extract was expressed in anti pernicious anemia (APA) units. With the introduction of microbiological assay methods, the LLD unit was defined in terms of increased growth produced by vitamin B₁₂ in cultures of *Lactobacillus lactis* Dorner. After pure crystalline vitamin B₁₂, cyanocobalamin, was shown to have these two activities (APA and LLD), the weight of vitamin B₁₂ equivalent to each of these units was carefully established. One microgram of crystalline vitamin B₁₂ is equivalent to one APA unit or to eleven thousand LLD units. Doses and concentrations of vitamin B₁₂ are now expressed in terms of weight of cyanocobalamin. Because of the extremely low levels which can be detected, the unit of weight may be the microgram (0.000001 Gm), the millimicrogram (0.000000001 Gm) or even the micromicrogram (0.000000000001 Gm).

The method of choice for the assay of vitamin B₁₂ depends upon the purpose of the determination and the nature of the material to be analyzed. Each type of assay offers certain advantages and disadvantages. Of the microbiologic methods, the *Ochromonas* assay is best suited for determination of true vitamin B₁₂ activity. The microbial assays, however, are more practical for routine purposes when the presence of pseudovitamin B₁₂ is not a complicating factor.

Many substances have been discovered which resemble the cobalamins in some properties but which lack vitamin B₁₂ activity in man and animals. At present, the most reliable methods for the determination of vitamin B₁₂ activity are the radioisotope tracer assay and the biologic assays based on animal growth. When identity as a cobalamin is assured, however, many alternative methods can be selected, based on precision, speed, and concentration of sample.

CHEMICAL METHODS

Most chemical methods for the analysis of vitamin B₁₂ can be used successfully only on samples which are moderately concentrated and free of interfering substances.

Spectrophotometric Assay

Cyanocobalamin may be identified and assayed by its light absorption spectrum in the visible and ultraviolet regions. The United States

Pharmacopeia, Fifteenth Revision, specifies a spectrophotometric method in which the assay may be calculated from the absorbance at 361 millimicrons if the absorbance maxima and their ratios are within the specified limits ³ The absorptivity of cyanocobalamin at 361 millimicrons is 207 The U S P test entitled "Pseudo cyanocobalamin" determines the presence of any similarly absorbing substances which are extractable from cresol carbon tetrachloride with 25 per cent sulfuric acid This extraction procedure provides, in some measure, the specificity inherent in the extraction methods of the radioisotope tracer assay discussed below

In the absence of interfering substances, the spectrophotometric method can be used for assaying triturations, solutions and other simple pharmaceutical formulations to which cyanocobalamin has been added The direct spectrophotometric method is speedy and accurate for such assays and may be used to determine as little as 50 micrograms of cyanocobalamin Its usefulness is limited, however, in the assay of products containing vitamin B₁₂ like compounds or other colored interfering substances

Countercurrent Distribution Assay

The distribution coefficient of cyanocobalamin between mutually saturated water and benzyl alcohol is 1.2 A multiple extraction system of identification and assay based on this property has been described ³³ The method is especially suited to the determination of the cyanocobalamin content of crystalline vitamin B₁₂, and similar products of relatively high purity

Radioisotope Tracer Assay

A combination of selective extractions and adsorptions has been described which purifies cyanocobalamin solutions sufficiently for spectrophotometric determination ³⁴ The fraction of the original cyanocobalamin recovered in the final extract is determined by use of tracer cyanocobalamin containing radioactive Co⁶⁰, thus permitting calculation of the amount of cyanocobalamin in the unpurified sample

The cobalamin assay, described in the First Supplement to U S P XV³⁵ and the Second Interim Revision Announcement for N F X,³⁵ is a tracer method and is highly specific for cobalamins which are convertible to cyanocobalamin ³⁶ Many interfering substances, including pseudovitamin B₁₂, are removed by washing a cresol carbon tetra

chloride solution with dilute sulfuric acid. An alumina resin column removes both ionic and nonionic impurities, allowing the electrically neutral cyanocobalamin to pass through. If treatment with cyanide is omitted, the cobalamin assay can be made specific for cyanocobalamin. Two hundred to 500 micrograms of total cobalamins are required. Although the cobalamin assay requires special equipment and procedures and is rather time-consuming, it is not difficult to apply routinely. It is unique in its combination of high accuracy and high precision. It can be used to measure cyanocobalamin specifically, even in crude extracts of low potency, as well as cobalamin concentrates, liver extracts, and vitamin B₁₂ with intrinsic factor concentrates.

Colorimetric Assays

Soon after the discovery of crystalline vitamin B₁₂, the important role of cyanide in its chemistry was found.²⁷ An assay method based upon the liberation of cyanide from cyanocobalamin by chemical reduction or by photolysis with visible light was developed.²⁸ The cyanide is carefully collected and measured by a highly sensitive colorimetric procedure. By measurement of cyanide liberated before and after treatment of the sample with an excess of cyanide ion, this method can be used to determine cyanocobalamin as well as other cobalamins which are readily convertible to cyanocobalamin. The colorimetric cyanide method has been used to determine the cyanocobalamin content of many pharmaceutical formulations or the total cobalamin content of liver extracts containing large quantities of substances which interfere with the spectrophotometric measurements of vitamin B₁₂. Because it is one of the most sensitive chemical methods for cyanocobalamin, it can be used to determine as little as 0.5 microgram with precision. Although suitable for assaying formulations known to contain vitamin B₁₂, it is not specific because other non-vitamin materials, for example pseudovitamin B₁₂, also liberate cyanide.

The dicyanide assay²⁹ is based upon the difference between the visible spectrum of cyanocobalamin and its purple dicyanide complex formed upon addition of excess cyanide. Following preliminary extraction with suitable solvents to eliminate extraneous pigments and to concentrate the vitamin B₁₂, this procedure is applicable to the measurements of the total cobalamin content of crude concentrates and culture filtrates.

BIOLOGICAL METHODS

Biological assay methods for vitamin B₁₂ are hampered by the difficulty of satisfactorily depleting the test animals. This difficulty is encountered because the young growing animals are endowed by the mother with adequate reserves. This has been partially overcome by including stress factors in the diet which intensify the depletion⁴⁰⁻⁴¹ or by the feeding of special diets which increase the requirements for vitamin B₁.⁴²⁻⁴³ These methods are time-consuming and not highly precise but, with the exception of the anti pernicious anemia test in human subjects, the growth responses in animals are probably the most specific measure of vitamin B₁₂ activity and availability.

In the chick assay,⁴⁴⁻⁴⁵ the chicks used must be deficient in vitamin B₁₂. Duplicate groups of chicks are fed vitamin B₁₂ free diets supplemented either with known amounts of vitamin B₁₂ or with the unknown sample. After the assay period, a standard growth response curve is prepared from which the activity of the unknown is estimated.

The rat assay⁴⁶⁻⁴⁸ uses weanling rats from vitamin B₁₂ depleted mothers. After seven or more days on a vitamin B₁₂-free diet, the rats receive the test diets for a two week period. The assay is based on the average weight gain during this period.

MICROBIOLOGICAL METHODS

The most sensitive methods for detecting and estimating vitamin B₁₂ depend on its stimulation of the growth of microorganisms. Highly developed methods based on several species of organism have been described.⁴⁷ The role of vitamin B₁₂ in the metabolism of microorganisms has been studied intensively and many vitamin B₁₂ like substances have been discovered.⁴⁸

Fortunately the problem of specificity is of little concern in the assay of pharmaceutical formulations containing only cyanocobalamin. The choice of microbiological assay methods can be made on the basis of precision, experience and convenience, but there is no general agreement on the superiority of any of the many methods.

Many of the organisms suitable for assaying cyanocobalamin can be grown either in fluid media in tubes or in agar media on plates, making possible a multitude of permutations. In general, plate methods are less sensitive, often by a factor of 1,000 or more, but are easier to use and may be more precise. Tube methods using sensitive organisms may measure as little as one micromicrogram of vitamin

B₁₂ The preparation of assay samples of most pharmaceutical formulations usually involves simple dilution. Extraction of vitamin B₁₂ from crude materials and its release from 'bound' forms may require special treatment.⁴⁷

Lactobacillus Assays

Many methods differing in details of procedure, are based on growth of lactobacilli. The vitamin B₁₂ activity assay described in USP XV is a tube assay based on the growth of *Lactobacillus leichmannii*.⁴⁸ This method has received a great deal of collaborative study and gives results which can be duplicated in different laboratories. Although not specific for cobalamins, it is highly sensitive (0.01 microgram per cc) and may be replicated to obtain good precision. It is the method of choice for many formulations containing cyanocobalamin. The method, as described, measures turbidity due to increased growth, but the Lactobacilli are acid producers and titrimetric procedures for measuring increased acid production can also be used.

The earliest microbiological assay method for vitamin B₁₂ was based on the growth of *Lactobacillus lactis* Dorner.⁴ The LLD assays, especially those employing plate methods, are still widely used.

Escherichia coli Assay

Assays based on the growth of an *Escherichia coli* mutant have been widely used, both by the tube method (sensitivity 0.5 millimicrograms per cc) and the cup plate method (sensitivity 5.0 millimicrograms per cc).⁴⁹

Euglena gracilis Assay

An assay for vitamin B₁₂ based on the growth of *Euglena gracilis* has also been developed. It can be adapted to tube or plate assay methods with an incubation period of 8 to 10 days.⁵¹ It is thought by some to be more sensitive than the *Lactobacillus leichmannii* assay, since as little as one micromicrogram of cyanocobalamin per cc can be determined.

Ochromonas malhamensis Assay

An assay based on the growth of the chrysomonad, *Ochromonas malhamensis* which responds almost specifically to cyanocobalamin has been described.⁵² Because of its slow growth and because few laboratories are familiar with cultures of the organism, this assay method has not come into general use.

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Part 2

Nutritional, Metabolic,
Physiologic, Pharmacologic
and
Clinical Information

THE NUTRITIONAL ROLE OF VITAMIN B₁₂

Vitamin B₁₂ has been found to be essential for the growth of many microorganisms. Among these microorganisms are *Lactobacillus lactis* Dorner,¹ *Lactobacillus leichmannii*,² a mutant strain of *Escherichia coli*,³ the chlorophyll-containing protozoan, *Euglena gracilis*⁴ and the chrysomonad, *Ochromonas malhamensis*.⁵

Of the vitamin B₁₂ dependent microorganisms known, the lactic acid bacteria are the most sensitive and *Ochromonas malhamensis* the most specific in their requirement for vitamin B₁₂. Methionine, desoxy-ribosides, or pseudovitamin B₁₂ are capable of supplying the vitamin B₁₂ requirements for most of these organisms. None of these compounds are active, however, for *Ochromonas malhamensis*.

Table 1 The Vitamin B₁₂ Activity Content of Some Common Foods

Food	Vitamin B ₁₂ Activity (mcg/Kg)
Milk products	
Whole milk	3.5 (per liter)
Evaporated milk	1.3 (per liter)
Condensed milk	3.5 (per liter)
Whole milk powder	10.26
Skim milk powder	25.40
Cheese	
American	6
Swiss	9
Cream	2
Canned meats	
Beef with juice	10.25
Pork with juice	10.20
Veal with juice	10.20
Lamb with juice	20.50
Beef liver	200.600
Cured meats	
Bologna sausage	17
Pressed ham	11
Frankfurter	18
Pork sausage	15
Sea foods	
Fillet of sole	13
Haddock	11
Scallops	7
Eggs (whole)	1.5 per egg

In the following foods microbial growth response was equivalent to less than 1 mcg per Kg. of Vitamin B₁₂ activity: Canned carrots, beets, peas, green beans, canned soups—tomato, vegetable, chicken, lamb, vegetable, cereal products—white flour, whole wheat flour, white bread.

Not only do some microorganisms require vitamin B₁₂, but many are capable of synthesizing relatively large quantities of the vitamin.⁶ Cobalt markedly stimulates the biosynthesis of the vitamin by microorganisms grown in common bacteriologic media.⁷ Detectable quantities of vitamin B₁₂ activity are found in animal proteins and fermentation products. Plant proteins appear to be devoid of the vitamin. Meats, especially liver, and eggs, milk, cheese and fish are among the richer sources of the vitamin. Table 1 indicates the content of the vitamin B₁₂ activity in several groups of common foodstuffs.⁸ The vitamin B₁₂ activity content of these foods was determined by microbial assays employing the lactic acid bacteria. Although these microorganisms respond to materials other than vitamin B₁₂, the values obtained agree fairly well with those obtained by other methods.

During the early investigations on liver and its relation to pernicious anemia, nutritionists also were engaged in determining the nutritional requirements of domestic animals. Studies on chicks and rats firmly established that optimal growth in these species was a function of animal protein intake. The essential nutrient present in animal protein was designated animal protein factor (APF).⁹ Vitamin B₁₂ was shown in chick experiments to possess a high degree of APF activity.¹⁰ Vitamin B₁₂ was also found essential for normal growth of the rat,¹¹ the mouse¹² and the pig.¹³

It is now firmly established that vitamin B₁₂ is an essential growth factor for certain microorganisms, for laboratory animals, and for poultry and nonruminant farm animals. Although it is also essential to human nutrition, the minimum daily requirements of vitamin B₁₂ have not been established.

THE METABOLIC ROLE OF VITAMIN B₁₂

Although the physiologic effects of vitamin B₁₂ on blood formation, neural function and the maintenance of growth in young animals are now well established, these effects cannot as yet be fully explained in terms of specific metabolic functions of the vitamin. Extensive data have been collected, however, on the influence of vitamin B₁₂ on a number of biochemical reactions which are concerned with intermediary metabolism.

Indirect evidence indicates that vitamin B₁₂ plays a role in the synthesis of nucleic acids. Vitamin B₁₂ deficiency in rats decreases the

ribonucleic acid content of the liver, spinal cord and cervical ganglia ¹⁴ Although both the ribonucleic and desoxyribonucleic acids per gram of liver are significantly reduced in the deficient animals, ¹⁴ ¹⁵ there is no change in the amount of nucleic acid per cell ¹⁵ It appears, therefore, that the synthesis of nucleic acid is markedly reduced in vitamin B₁₂ deficiency and this causes a decrease in cell division The effect of vitamin B₁₂ deficiency on nucleic acid synthesis and cell division has also been demonstrated in human bone marrow cells from pernicious anemia patients ¹⁶ Considerable decrease in the synthesis of thymine was observed in marrow cells, which is consistent with other data on the specific function of vitamin B₁₂ in the synthesis of this pyrimidine base ¹⁷

Rats receiving vitamin B₁₂ will grow on diets deficient in choline and methionine, but containing homocystine This suggests that the vitamin has a function in the metabolism of labile methyl groups ¹⁸ Further evidence of this function is provided by the observation that vitamin B₁₂ will prevent the characteristic hemorrhagic kidney syndrome which develops in weanling rats fed a choline free diet ¹⁸ Studies with vitamin B₁₂-deficient rats and baby pigs fed precursors containing C¹⁴ labeled methyl groups have shown that vitamin B₁₂ enhances the synthesis of choline and methionine methyl groups from formate, from the β -carbon of serine and the α -carbon of glycine ¹⁹⁻²¹ The available evidence also shows, however, that vitamin B₁₂ is not involved in the actual mechanism of transmethylation from betaine to methionine and from methionine to choline ²²⁻²⁵

Vitamin B₁₂ deficiency in rats, as well as in patients with pernicious anemia, causes a marked diminution in the blood levels of soluble sulfhydryl compounds ²⁶ The glutathione content of liver and blood of vitamin B₁₂-deficient rats is significantly diminished ²⁷ Some of the effects of vitamin B₁₂ on carbohydrate metabolism may be mediated through glutathione, which as the prosthetic group of glyceraldehyde-3 phosphate dehydrogenase, is presumably essential in the glycolytic pathway Homocystine, on the other hand, is essential as an acceptor of methyl groups in the process of labile methyl group synthesis and transfer ¹⁸

Vitamin B₁₂ deficiency in rats and chicks leads to a marked increase in the coenzyme A content of the liver and kidneys ²⁸ ²⁹ The increase occurs chiefly in the sulfhydryl (reduced) form of the coenzyme The increase is probably a physiologic adaptive mechanism and may be

involved in the effects of vitamin B₁₂ deficiency on carbohydrate and fat metabolism ³⁰

It has been postulated that vitamin B₁₂ may be involved in the conversion of folic acid to folinic acid ³¹ It has been shown that this conversion is decreased in liver homogenates from vitamin B₁₂ deficient hens ³² There is also some indication that the conversion of folic acid to folinic acid is decreased in pernicious anemia patients in relapse ³³ Since the conversion of folic acid to folinic acid by chick liver homogenates is enhanced by homocysteine, ³⁴ it is possible that the action of vitamin B₁₂ is an indirect one which concerns the increased reduction of homocysteine to homocystine

On the basis of growth studies in animals it has been shown that vitamin B₁ has some general effects on animal metabolism In hyperthyroid rats, it has a protein sparing action This does not prevent weight loss, however, for protein is spared at the expense of other body constituents ³⁵ Vitamin B₁₂ also permits better utilization of nitrogen in animals fed a diet high in soybean protein ^{36 37} Since the methionine content of soybean protein is low, this effect is probably related to the methionine sparing activity of the vitamin rather than to a direct effect of vitamin B₁₂ on protein synthesis

Some of the increased growth of animals receiving vitamin B₁₂ has been ascribed to an increase in appetite ^{38 39} This view is supported by the finding that the protein catabolic action of cortisone in rats is counteracted by vitamin B₁₂ under ad libitum feeding conditions, ⁴⁰ but is not affected when food intake is restricted ⁴⁰ Most of the increase in weight gain in young rats fed a plant protein diet supplemented with vitamin B₁₂ can be accounted for by gain of fat rather than protein ⁴¹

Some recent investigations in animals indicate that vitamin B₁₂ is concerned with the metabolism of carbohydrates It has been shown that the catabolism of fructose by rat liver homogenates is enhanced in animals receiving vitamin B₁₂ ⁴² It has also been shown in rats that the feeding of lactose ⁴³ or the injection of glucose ⁴⁴ leads to an increased requirement for vitamin B₁ Vitamin B₁₂ deficiency in chicks leads to an increase in blood glucose, while the increase in blood glucose obtained by feeding high levels of glycine, glutamic acid or methionine is enhanced by the administration of vitamin B₁₂ This suggests that the vitamin may function in the conversion of certain glucogenic amino acids into glucose ⁴⁵

Vitamin B₁₂-deficient rats on a high-carbohydrate, low fat diet rapidly lose weight, develop hyperglycemia and show a decrease in carcass phospholipids, which suggests that these animals have partially lost their ability to transform carbohydrates into lipids. In accord with this suggestion is the observation that abnormally small amounts of phospholipids are found in the tissues of vitamin B₁₂-deficient rats and in the blood of patients with pernicious anemia in relapse.⁴¹ The utilization of high dietary fat intake in chicks is improved following the administration of vitamin B₁₂,⁴² indicating that vitamin B₁₂ may be required for the metabolism of fat.

Although many investigations have shown that vitamin B₁₂ has a marked influence on animal growth, and certain evidence indicates that the vitamin aids in the utilization of proteins, carbohydrates and fats, the exact mechanism by which this is accomplished is still unknown. Experiments in rats have shown that vitamin B₁₂ will counteract the effects of excess production of or the administration of thyroid hormone⁴³ and adrenocortical hormones.⁴⁴ It has not been determined, however, whether there is a direct physiologic relation between vitamin B₁₂ and these hormones or whether these observed effects are due to the indirect influence of the vitamin on some phases of intermediary metabolism.

PHYSIOLOGY AND PHARMACOLOGY OF VITAMIN B₁₂

Vitamin B₁₂ is now known to be identical with the extrinsic or erythrocyte maturing factor of Castle,⁴⁵ which is essential for normal bone marrow erythropoietic activity in man. In the absence of the erythrocyte maturation factor, as in pernicious anemia and other macrocytic anemias, red cell maturation progresses abnormally and is arrested at a megaloblastic stage of development. Vitamin B₁₂ is also concerned with neural function and the maintenance of nerve cells and is required to sustain normal growth in young animals. The sources of vitamin B₁₂ for human nutrition are foods of animal origin such as meats, milk and eggs.

Vitamin B₁₂ and Intrinsic Factor

Vitamin B₁₂ in foods is very poorly absorbed by human beings unless intrinsic factor is present at the same time. Extensive studies⁴⁶⁻⁵¹ have shown that an extrinsic factor present in the diet, which is now

known to be a vitamin B₁₂ active substance, is required for normal hemopoiesis in man. Intrinsic factor, produced in the stomach, is found in abundance in normal gastric juice. Its function is to facilitate the passage of vitamin B₁₂ across the intestinal mucous membrane. Pernicious anemia occurs in human beings who lack intrinsic factor and, therefore, are unable to absorb vitamin B₁₂ from dietary sources in amounts adequate for normal hemopoietic function. Although considerable progress has been made in attempts to isolate and purify the intrinsic factor,⁵²⁻⁵⁵ its chemical structure and the exact nature of its action in facilitating the absorption of vitamin B₁₂ are still unknown.⁵⁶

It was originally postulated that the intrinsic factor interacted in some manner with vitamin B₁₂, probably in the small intestine, and that the product of this interaction was present in blood serum as hemopoietic factor.⁵⁷ Despite extensive studies to determine the nature of the interaction between vitamin B₁₂ and intrinsic factor, information is still incomplete concerning the exact site of the interaction, if any, and the conditions controlling it.

Some species specificity of intrinsic factor is indicated by studies which showed that intrinsic factor of human or porcine origin, which is active in pernicious anemia patients, did not facilitate the intestinal absorption of vitamin B₁₂ in gastrectomized rats, but rat intrinsic factor was fully active.^{58, 59}

Absorption and Excretion of Vitamin B₁₂

If the amounts of vitamin B₁₂ ingested are small and intrinsic factor is present, most of the absorption takes place with the aid of this factor.⁶⁰ Over a certain range, there is a stoichiometric relationship between the amount of vitamin B₁₂ absorbed by pernicious anemia patients and the amount of intrinsic factor given.⁶¹ When large amounts of vitamin B₁₂ are given, however, some direct intestinal absorption of the vitamin occurs without mediation of intrinsic factor. The direct absorption assumes greater importance as the level of intake of vitamin B₁₂ is increased.⁶² The amount absorbed by direct diffusion across the intestinal barrier, however, is very small when relatively large amounts of the vitamin are administered orally to normal individuals or to pernicious anemia patients receiving adequate intrinsic factor.^{62, 63} The intestine has a limited capacity to absorb vitamin B₁₂ given in single oral doses even in the presence of an excess of intrinsic factor.⁶⁴ The amount of vitamin B₁₂ absorbed and

the level at which intestinal absorption is restricted varies greatly in normal subjects and in patients with pernicious anemia^{61 62 63}

After rectal administration of very large doses, the increase in serum concentrations and urinary excretion of vitamin B₁₂ and the hematologic responses in pernicious anemia patients indicate significant but variable absorption⁶² Direct absorption of vitamin B₁₂ by the nasal mucosa following the instillation or insufflation of large therapeutic doses in pernicious anemia patients has been observed,^{64 65} and the urinary excretion patterns are similar to those observed after parenteral injections⁶⁶

Vitamin B₁₂ containing radioactive cobalt has been used quite successfully, in tracer studies to estimate the intestinal absorption of the vitamin⁶⁷ When the oral dose of labeled vitamin B₁₂ is very small (0.5 microgram) more than 50 per cent of the administered dose is absorbed in normal persons and less than 15 per cent in pernicious anemia patients, as determined by fecal elimination and hepatic deposition of radioactive cobalt⁶⁸ When the oral dose of labeled vitamin B₁₂ is increased to about 50 micrograms, as much as 70 to 80 per cent of the radioactivity may be eliminated in the feces, indicating very little absorption even in normal persons^{70 71}

The absorption of physiologic amounts of the vitamin has been similarly estimated by measuring over a fixed period the radioactivity of the urine following oral administration of small doses of radioactive vitamin B₁₂ The radioactive vitamin B₁₂ is flushed from the body by a subsequent large intramuscular dose of nonradioactive vitamin B₁₂ Under these conditions all of the radioactivity found in the urine must be derived from the vitamin B₁₂ absorbed from the gastrointestinal tract⁷ Renal disease must be considered in interpretation of the results of this test since individuals with kidney involvement may show a low rate of vitamin B₁₂ excretion, sometimes in the range shown by pernicious anemia patients Such individuals, however, unlike pernicious anemia patients, do not show increased excretion with administration of intrinsic factor, and they continue to excrete the radioactive vitamin beyond the first 24 hours after the flushing dose⁷²

Labeled vitamin B₁₂ has also been used to study the distribution and excretion of vitamin B₁₂ after parenteral and oral administration to rats After subcutaneous injection, approximately half of the radioactivity was rapidly excreted in the urine, and about 6 per cent in the

feces The remainder was distributed throughout the body in various organs After oral administration, 81 per cent was eliminated in the feces, and about one per cent in the urine ⁷¹

After absorption, vitamin B₁₂ appears in the blood stream and circulates to the various sites of its metabolic activity Normal human serum levels of vitamin B₁₂ vary greatly and may range from 100 to 900 micromicrograms per cc ⁶² with the major portion apparently bound to the alpha globulin fractions of the serum proteins ⁷⁵ The serum concentrations in patients with pernicious anemia are definitely below the normal range After treatment with vitamin B₁₂, the serum concentrations return to normal or above and such levels can be maintained by adequate therapy When therapy is discontinued, the serum concentration of vitamin B₁₂ gradually declines as relapse occurs The bone marrow, however, usually remains normoblastic as long as the serum levels are above 100 micromicrograms per cc ⁶⁶

Parenterally administered vitamin B₁₂ is rapidly absorbed and appears in the blood serum in both free and bound form Following single injections in normal persons or in patients with pernicious anemia in relapse, serum levels of vitamin B₁₂ rise rapidly, reach a maximum within one hour and decline gradually over a period of 24 hours or more Most of the injected vitamin is rapidly excreted in the urine The proportion of the dose excreted increases with the amount injected ⁷⁶ The free form of the vitamin is readily excreted by the kidney

Following single oral doses of vitamin B₁₂, little or no change in serum levels has been observed unless the amount given is 500 micrograms or more ⁷¹ With single oral doses of 1,000 to 3,000 micrograms or more, definite but variable increases in the serum concentrations have been demonstrated ^{62, 76} After these large oral doses there is an increase in urinary excretion of the vitamin which is considerably less than that observed after intramuscular injection of amounts that produce equivalent serum concentrations ⁷⁸ This lower urinary excretion is associated with greater binding of the vitamin by serum protein after oral than after parenteral administration ⁶²

Toxicity

Although vitamin B₁₂ is probably the most active biological principle known, it is not toxic when administered orally or parenterally No manifestations of toxicity were observed in studies with mice, rats and guinea pigs Intraperitoneal or intravenous injections of crystal

line vitamin B₁ in quantities as high as 1.6 Gm. per kilogram in mice produced no toxic effects. No toxic effects were produced in rats and guinea pigs with intraperitoneal injections of 100 mg. per kilogram.⁷⁷

No acute or chronic toxic effects have been observed in man even after the parenteral administration of very large doses of pure crystalline vitamin B₁. Doses as large as 1,000 micrograms or more have been administered by subcutaneous or intramuscular injection without local irritation.⁸⁰ Daily intravenous injection of doses as large as 3,000 micrograms were painless and nontoxic and no allergic side reactions were observed.⁸¹ Single oral doses as large as 10,000 micrograms administered to pernicious anemia patients have produced no apparent toxic effects.⁸ During continued oral therapy with doses of 1,000 micrograms of crystalline vitamin B₁₂ per week for periods of three to five years, no adverse effects were observed.⁸⁰

Although a few allergic reactions were observed following the therapeutic use of some of the early concentrates⁸¹ and crystalline preparations of vitamin B₁₂,⁸⁰ hypersensitivity reactions have not occurred following administration of the pure crystalline vitamin.⁸⁰ In a group of patients who showed allergic reactions after therapeutic liver injections, with strong positive reactions to liver extracts, skin tests with pure vitamin B₁ were negative.⁸²

CLINICAL USES

Since its discovery, vitamin B₁ has been found useful in an increasing number of clinical conditions. Its efficacy in pernicious anemia is established beyond question and it has often been effective in other macrocytic anemias. It has also found a place in the treatment of various neurologic disorders. These include not only the neurologic disturbances accompanying pernicious anemia and diabetes mellitus but also trigeminal neuralgia, herpetic neuralgia and possibly other neuropathies.

Other clinical studies indicate the potential usefulness of the vitamin in a variety of physiologic states or clinical conditions. Certain skin disorders and liver disorders have responded in some cases. The increased need for vitamin B₁ in many nutritional states is becoming increasingly apparent. It is being used as a dietary supplement in debilitated or elderly patients, and it has been useful in promoting growth in ill or undernourished children. In such conditions the beneficial effects of the vitamin on appetite have been extremely important.

Extensive investigation has indicated that vitamin B₁₂ is effective not only by the parenteral route but also in large oral doses and, in some cases, by nasal insufflation. Pure crystalline vitamin B₁₂ does not produce local irritation or sensitivity reactions when administered parenterally, nor does it cause gastric irritation when given orally.

MACROCYTIC ANEMIAS

Vitamin B₁₂ deficiency is involved in certain anemias resulting from disturbance of the hemopoietic function of the bone marrow. If the vitamin is lacking in the diet for a prolonged period, or if it is inadequately absorbed or utilized, erythrocyte maturation is arrested, resulting in macrocytosis and a decline in red cell count. In addition, megaloblasts may appear in the bone marrow and peripheral blood. Megaloblastosis is characteristic of pernicious anemia and occurs frequently in the other macrocytic anemias in which vitamin B₁₂ deficiency is involved.

Following vitamin B₁₂ therapy in such anemias, the hemopoietic functions of the bone marrow are promptly restored to normal. Erythrocyte maturation is hastened, the per cent of reticulocytes in the peripheral blood is increased temporarily, megaloblasts are greatly reduced in number, and are replaced by more mature cells. The red cell count gradually returns to normal.

Addisonian Pernicious Anemia

Pernicious anemia is a chronic, relapsing disease, caused by a conditioned deficiency of vitamin B₁₂.⁸³ It is a fatal disease unless the deficiency is corrected. The deficiency results from failure of the gastric mucosa to produce sufficient intrinsic factor,⁸¹ the substance essential for absorption of extrinsic factor, which is now known to be vitamin B₁₂ or a B₁₂ active substance. Patients lacking intrinsic factor are unable to assimilate the vitamin even when it is present in normal amounts in the diet. The amount of intrinsic factor present in pernicious anemia patients probably varies,⁸⁴ but it is inadequate for absorption of the required amounts of vitamin B₁₂ in all these patients.⁸² The resulting disease involves primarily the hemopoietic system, the nervous system, and the alimentary tract. Characteristic pathologic changes involve the tongue, gastric mucosa, bone marrow, peripheral blood, and nervous system. Achlorhydria, a characteristic finding is evidence of the defect in the gastric mucosa which causes the disorder.

The red cell count is reduced, in some cases to less than 10 per cent of normal. Although hemoglobin synthesis is not affected the great reduction in red cells means that the percentage of hemoglobin present in the blood is also greatly reduced. The average diameter of the erythrocytes is increased and many very large cells called macrocytes are found. Reticulocytes are increased to about 2 or 3 per cent of the red cell population. Megaloblasts are characteristically present in the blood. The bone marrow is megaloblastic and hyperplastic.

Neurologic changes range from peripheral nerve degeneration to degeneration in the posterior and pyramidal tracts of the spinal cord and in the subcortical areas in the motor region of the brain. Such changes lead to a variety of symptoms, including paresthesias, difficulties in walking, incoordination, loss of position sense, and mental disturbances.

Other common symptoms of the disease include weakness, anorexia, pallor, and gastrointestinal disturbances. Soreness of the tongue which may become smooth because of papillary atrophy, is common. Liver and spleen enlargement are sometimes present. The skin may take on a lemon yellow tint.

The treatment of choice is vitamin B₁₂, which promptly produces complete hematologic remission and neurologic improvement.⁸⁵ The first detectable response is a dramatic increase in the number of reticulocytes which rise to a maximum within a week after the start of therapy. Thereafter the reticulocytes rapidly mature and the erythrocyte count gradually rises. Erythrocytes and hemoglobin usually are normal within four to six weeks. Bone marrow is converted from a megaloblastic to a normoblastic state within 48 to 72 hours. Within a few days after start of treatment appetite and sense of well being improve. Glossitis subsides and after several weeks lingual papilla regeneration takes place. Neurologic complications respond more slowly to vitamin B₁₂ therapy but will subside under continued treatment unless irreversible changes in the brain or spinal cord have occurred.^{85 86 87} (The neurologic complications of pernicious anemia and their response to vitamin B₁₂ therapy are further discussed under **NEUROLOGIC DISORDERS**.) Patients with pernicious anemia can be maintained in good health as long as adequate vitamin B₁₂ therapy is continued.^{87 88}

The efficacy of vitamin B₁₂ administered by the parenteral route for pernicious anemia has long been established. It is also effective when

given orally with intrinsic factor.⁸³ One study in pernicious anemia patients, however, has indicated that this method may lose its efficacy after prolonged use of heterologous intrinsic factor, though patients will still respond satisfactorily if human intrinsic factor is used. The authors postulate that some failures with oral treatment may be due to this phenomenon.⁸⁹

Formerly it was thought that intrinsic factor was essential to effect absorption of orally administered vitamin B₁₂.^{83, 88, 91} Investigation has shown, however, that even without intrinsic factor, very large oral doses may produce a satisfactory hematologic and neurologic response which can be sustained by continued therapy.^{87, 9, 93}

Anemia Following Gastrectomy

Failure to secrete intrinsic factor with resultant lack of vitamin B₁₂ absorption not only occurs in pernicious anemia but may also follow gastrectomy or accompany malignant disease of the stomach. Consequently a macrocytic anemia closely resembling pernicious anemia⁹⁴⁻⁹⁶ frequently follows gastrectomy and is sometimes associated with gastric carcinoma. In the latter case this may be the result of interference with intrinsic factor secretion by the carcinomatous growth. Some investigators, however, suggest that lack of intrinsic factor may precede rather than follow development of gastric cancer.⁹⁷ In this connection the increased incidence of the disease in pernicious anemia patients and in nonanemic but achlorhydric patients has been reported and the authors believe that patients with achylia gastrica, as demonstrated by the Schilling urinary excretion test, probably have the same predilection for gastric cancer as those with pernicious anemia.⁹⁷

In totally gastrectomized patients, the reason for failure to secrete intrinsic factor is loss of the fundal portion of the stomach.⁸⁵ Tests with small doses of radioactive vitamin B₁₂ show that this amount of the vitamin is not absorbed when administered to patients with total gastrectomy unless intrinsic factor is also administered.^{100, 99} Consequently in untreated patients the concentration of vitamin B₁₂ in the serum decreases progressively after operation.⁹⁸ In spite of this, the anemia usually does not appear for several years following the operation and neurologic symptoms may be even longer delayed.¹⁰⁰ Occasionally, spinal cord degeneration develops,¹⁰¹ even when anemia is not present.¹⁰ Both anemia and neurologic symptoms in these patients respond well to treatment with vitamin B₁₂.^{100, 103, 104} Whether total

gastrectomy is done for the treatment of carcinoma or peptic ulcer, vitamin B₁₂ should thereafter be administered regularly to prevent the development of macrocytic anemia, which otherwise frequently follows 96 98 100 103

Macrocytic anemia has also been reported after partial gastrectomy and after gastroenterostomy,¹⁰⁶⁻¹⁰⁷ though its incidence is not as high as it is following complete gastrectomy.¹⁰⁷ Regular administration of vitamin B₁₂ is therefore advisable following these operations

Anemia Accompanying Intestinal Strictures and Anastomoses

A macrocytic anemia which responds to vitamin B₁₂ is occasionally associated with intestinal strictures or anastomoses.^{108, 109} Symptoms are like those in pernicious anemia and the blood findings are identical with those in pernicious anemia both in morphology and degree of reduction of the red cell count. The bone marrow is hyperplastic and megaloblastic. Achlorhydria, however, is not characteristic and intrinsic factor is usually present. Although the exact mechanism of the production of this anemia is not known, it has been postulated¹¹⁰ that intestinal stagnation due to stricture permits abnormal bacterial growth in the small intestine, or short circuit of the intestine occurs, either of which may result in impaired utilization of vitamin B₁₂. Relief of symptoms and satisfactory hematologic response have been observed in this type of macrocytic anemia following the administration of vitamin B₁₂, whether or not the intestinal anomaly has been corrected by surgical measures

Fish Tapeworm Anemia

The symptoms and hematologic findings in some patients infested with fish tapeworm (*Diphyllobothrium latum*) are almost indistinguishable from those in pernicious anemia.¹¹¹ The macrocytic anemia is caused by subnormal absorption of vitamin B₁₂, or by competitive use of the vitamin by the parasite.¹¹¹ Although removal of the worm is the primary therapy, vitamin B₁₂ produces satisfactory hematologic and neurologic responses whether or not the worm has been passed 9, 111 113

Nutritional Macrocytic Anemias

The symptoms of nutritional macrocytic anemia, including glossitis and associated mucous membrane lesions, are similar to those in

pernicious anemia, and the hematologic findings are identical. Severe neurologic disturbances, however, are rare, also deficient or otherwise abnormal gastric secretions are not characteristic of the condition.⁸⁵ The syndrome is encountered in malnourished persons.

A deficiency of either vitamin B₁₂, or more frequently folic acid, or both, is the cause of this anemia.⁸⁵ Individuals who eat much carbohydrate but little protein or fat, either because of poverty, social habits, or religious beliefs, are subject to such deficiencies. The anemia is frequently found in association with other severe deficiency states such as beriberi and pellagra. Macrocytic anemias resulting from deliberate deprivation of dietary sources of vitamin B₁₂ have also been reported. Among the best examples of this are the Vegans, who eat no animal products. During the period of deprivation of vitamin B₁₂, their serum vitamin B₁₂ levels fall and they eventually develop paresthesias, menstrual disorders, pains of the spine and back, and finally anemia. The addition of vitamin B₁₂ to the diet alleviates these manifestations.¹¹⁴⁻¹¹⁷

A study of the serum vitamin B₁₂ levels in 6 patients with nutritional macrocytic anemia in India revealed that the values were significantly lower than in normal Indians.¹¹⁸ Some African patients, on the other hand, showed high serum vitamin B₁₂ levels and subsequently responded only to folic acid therapy.¹¹⁹

Vitamin B₁₂ given parenterally may produce a hematologic response in this disease and is advisable as the initial treatment if the diagnosis is doubtful.¹²⁰ The oral route may be satisfactory but parenteral administration is essential when gastrointestinal absorption has been impaired.⁸⁶

In many patients deficiency of vitamin B₁₂ and folic acid may be present simultaneously. In such patients administration of both vitamins may lead to more favorable results than would the administration of either vitamin alone. It is advisable to select vitamin B₁₂ as the initial therapeutic agent in cases in which the diagnosis is uncertain.⁸⁷

A megaloblastic anemia of nutritional origin is occasionally encountered in infants 3 to 18 months old. This disease has almost disappeared in the United States as a result of vitamin supplementation and better dietary habits. It occurs in children maintained entirely on a diet of goat's milk or of powdered cow's milk,¹²¹ and in those whose diets were not supplemented by required foods at the proper time.¹² Loss of appetite, intermittent fever, and pallor characterize the disease.

Hemic murmurs hepatic enlargement and, less consistently, spleno megaly may be found. The blood findings are identical with those in pernicious anemia except that leukocytosis may be present.

The etiology of this anemia is not certain but it is probably due to a multiple vitamin deficiency.¹²³ In some studies vitamin B₁₂ alone has produced satisfactory response,^{122, 124} but frequently it has been ineffectual and therefore should not be used as the sole therapeutic agent.^{88, 125}

Dimorphic anemias of nutritional origin in which a deficiency of vitamin B₁₂ or folic acid and a deficiency of iron coexist, may be macrocytic and hypochromic. An initial response may be obtained following administration of vitamin B₁₂ or folic acid but the peripheral blood findings may then change to those of a microcytic, hypochromic anemia. Although separate deficiencies of both vitamin B₁₂ and iron may thus be demonstrated, an erythropoietic response to iron therapy may not occur unless vitamin B₁₂ is also administered.

Vitamin B₁₂ is not indicated in the specific therapy of hemolytic anemia or the anemia of chronic blood loss, but it may be administered if the anemia is so prolonged that severe secondary symptoms such as anorexia, nausea, and impaired metabolic function are produced, thus creating a conditioned nutritional deficiency of vitamin B₁₂.

Anemias Associated with Gastrointestinal Disorders

Tropical and nontropical sprue, idiopathic steatorrhea and celiac disease are chronic relapsing diseases characterized by voluminous frothy stools and frequently by macrocytic anemia and a megaloblastic bone marrow. Neurologic changes may occur which are similar to, but less severe than those found in pernicious anemia. The etiology of these conditions is not fully understood, but in all of them gastrointestinal disturbances and voluminous frothy stools occur, resulting in impairment of vitamin B₁₂ absorption.^{88, 126}

Vitamin B₁₂, administered parenterally in doses larger than those given in pernicious anemia, has been successfully used in the treatment of these disorders.^{85, 88, 127, 128} These disorders are characterized by deficiencies of both folic acid and vitamin B₁₂ and both hemopoietic agents have been used in their treatment.¹²⁹ The parenteral route has been recommended as the most effective and practical for the administration of vitamin B₁₂,¹³⁰ since gastrointestinal function is impaired. Oral doses of vitamin B₁₂ however have been reported effective in some cases.^{131, 132}

Anemia of Pregnancy

In megaloblastic anemia of pregnancy, the blood and marrow changes are usually very similar to those in pernicious anemia. Lesions of the spinal cord are absent, however, and achlorhydria is not a consistent finding. The onset of the anemia is usually in the third trimester. It has been observed most frequently in women whose diets have been deficient, but does occur in women on apparently adequate diets often enough to raise some doubts as to its dietary origin.⁶

This anemia is far more serious than the iron deficiency anemia of pregnancy or the physiologic anemia due to increased plasma volume. The megaloblastic anemia occurs far less frequently than the other two types. There is, however, evidence that cases of hypochromic anemia in malnourished women may be accompanied by a macrocytosis due to vitamin B₁₂ deficiency. In one study of 100 anemic pregnant patients, living under depressed economic conditions with poor nutrition, 11 had hyperchromic macrocytic anemia, 30 had hypochromic microcytic anemia, and 59 had hypochromic anemia accompanied by macrocytosis. Though typical megaloblasts were found in only a few of these cases, the bone marrow was hyperplastic and contained giant stab cells and giant myelocytes. In most of the patients who developed this dimorphic anemia, the diet was protein deficient, and both the serum iron and vitamin B₁₂ concentrations were low. The vitamin B₁₂ levels, in fact, approached those in patients with megaloblastic anemia. The authors suggest that the typical morphologic picture of megaloblastic anemia failed to develop in these patients only because of the concomitant iron deficiency.¹³³

Vitamin B₁₂ administered alone has produced striking hemopoietic responses in cases of megaloblastic anemia of pregnancy associated with nutritional deficiency.^{134, 135} It has also been effective in combination with ascorbic acid, which has proved ineffective alone.¹³⁶ Folic acid has been more consistently effective than vitamin B₁₂.^{85, 137} However, even when folic acid deficiency is the chief causative factor, vitamin B₁₂ may enhance response to folic acid therapy and hasten recovery.

NEUROLOGIC DISORDERS

Because the neurologic manifestations of pernicious anemia frequently respond dramatically to vitamin B₁₂, many investigators have treated other neurologic disorders with this vitamin. Thus far, vitamin B₁₂ has

proved useful in the treatment of several neuropathies, including trigeminal neuralgia, postherpetic neuralgia, diabetic neuritis, and other painful syndromes of obscure etiology.¹³⁹ The results, though difficult to evaluate because of the subjective nature of the symptoms, were prompt and definite enough in some instances to indicate that the vitamin has a direct action on nerve tissue.

Neurologic Complications of Pernicious Anemia

From 70 to 95 per cent of patients with pernicious anemia manifest varying degrees of neurologic involvement.¹³⁹ The symptoms are primarily those of sensory tracts and nerves. Peripheral nerve degeneration causes numbness and tingling, and sometimes hyperesthesia of the soles of the feet. Damage to the posterior and lateral columns of the spinal cord can cause ataxia, absence of reflexes, and incoordination. Changes in motor nerves, which are now rare, can cause paralysis and muscular atrophy.

Mental changes also occur in many patients. These include irritability, impaired concentration, dullness, drowsiness, and depression. It has been pointed out in this connection that cerebral as well as spinal lesions accompany pernicious anemia, but are often unrecognized. The end result, if treatment is inadequate, may be a severe dementia.¹⁴⁰ Cerebral symptoms may precede the appearance of anemia or of spinal or peripheral nerve involvement.

Vitamin B₁₂ is essential for the prevention and treatment of the neurologic and mental complications of pernicious anemia. Prompt improvement occurs and complete relief can eventually be obtained with intensive vitamin B₁₂ therapy, except when treatment has been delayed until irreversible changes have taken place. Adequate vitamin B₁₂ maintenance therapy also will prevent neurologic relapse.^{63 80 87}

In connection with the neurologic manifestations of pernicious anemia, it should be emphasized that folic acid not only is less effective hemopoietically in pernicious anemia than liver extract or vitamin B₁₂, but it also fails completely to alleviate or prevent the neurologic complications. An even more important consideration is that the transitory hemopoietic effect of folic acid therapy may dangerously mask progression of the disease. It has become increasingly common to find pernicious anemia patients who, having been treated with folic acid alone or in conjunction with insufficient vitamin B₁₂, show a temporarily satisfactory hematologic response but then suddenly develop

severe neurologic symptoms⁶³ It has been suggested that by stimulating hemopoiesis when an acute vitamin B₁₂ deficiency exists, folic acid further depletes the body of vitamin B₁₂, thus leading to severe neurologic damage^{141 14} Because of this hazard, vitamin B₁₂ in adequate dosage should be the initial therapy whenever doubts exist about the etiology of a megaloblastic anemia⁶³

Diabetic Neuropathy

Varying degrees of neural involvement were frequently found in patients with diabetes mellitus Changes in peripheral nerves as well as in the autonomic and central nervous systems may develop when the disease is inadequately controlled The pathogenesis of these neuropathies is not clear It has been suggested that they may result from the primary metabolic disturbance characteristic of the disease or from obliterative changes in the vasa nervorum, or possibly from both these factors¹⁴³ Nutritional deficiency has also been suggested as a cause¹⁴⁴ In this connection it is of interest to note that urinary excretion tests of vitamin B₁₂ have demonstrated poor absorption of vitamin B₁₂ in many diabetics,¹⁴⁵ and it has also been noted that some patients are more sensitive to insulin after taking vitamin B₁₂¹⁴⁶ These observations may account for the fact that vitamin B₁₂ in large doses is often effective in treatment of the neurologic disturbances accompanying diabetes¹⁴⁷⁻¹⁴⁹ These include polyneuritis,¹⁴⁷ ataxia,¹⁴⁴ paresthesias,¹⁴⁸ and nocturnal diarrhea^{143 144 150} Impaired vibratory sense also has been reported to improve after treatment with vitamin B₁₂¹⁴⁹

Trigeminal Neuralgia

Vitamin B₁₂ administered parenterally in large doses promptly relieves trigeminal neuralgia in a significant proportion of patients⁶³ Complete relief of pain has occurred,^{151 153} lasting in some cases for as long as 8 months¹⁵¹ In some patients who have undergone nerve block or extirpation procedures pain relief has not occurred as promptly, but ultimate results have been equally good^{151 154} Such results suggest that treatment with vitamin B₁₂ is advisable in patients with trigeminal neuralgia before more drastic procedures are attempted

Herpetic Neuralgia

Large intramuscular injections of vitamin B₁₂ have alleviated the severe pain of herpes zoster in some cases which were resistant to

other forms of therapy ^{155 156} In certain patients, pain has disappeared within 24 hours, and the lesions have cleared within 48 hours ¹⁵⁷ In one case severe itching was alleviated by vitamin B₁₂ therapy ¹⁵⁸

Multiple Sclerosis

Because of the efficacy of vitamin B₁ in preventing or reversing the demyelinating effects of pernicious anemia, treatment with massive doses of the vitamin has been tried in many cases of multiple sclerosis. The results have been variable, moreover, the spontaneous remissions characteristic of the disease make the results difficult to evaluate. Striking benefit was observed after very large doses of vitamin B₁₂ ¹⁵⁹ in a few patients with epileptiform seizures, and difficulties in speech, swallowing and walking. In some patients definite improvement has been observed, even to the point of rehabilitation ¹⁶⁰ In other patients the improvements were only of a subjective nature, consisting principally of increased appetite and sense of well being ^{160 161}

Miscellaneous Neuropathies

Vitamin B₁ has been used with benefit in various neuropathies which have been resistant to other therapy ¹⁶²⁻¹⁶⁴ The data on its use in many of these syndromes, though too scanty at present to permit definite conclusions regarding its efficacy, indicate promising areas for further investigation.

Vitamin B₁₂ has been used with good results by several investigators in the treatment of syndromes accompanying alcoholism. Korsakoff's psychosis was extremely responsive to treatment with vitamin B₁ in a small group of patients ¹⁶⁵ and a few patients with delirium tremens were also successfully treated ¹⁶⁵ Several groups of patients with alcoholic neuritis have obtained prompt relief of pain after injections of vitamin B₁₂ ¹⁶⁶⁻¹⁶⁸

Other painful neuritic syndromes have also been reported to respond to very large doses of vitamin B₁₂. These include radiculitis, ¹⁵⁹ neuritis of the cranial nerves, ¹⁵⁹ and peripheral neuritis ^{159 170} In the symptomatic treatment of peripheral neuritis it was found that only the pain which arose from pathologic involvement of the entire sensory neuron including the ganglion cells, was ameliorated by vitamin B₁₂ therapy ¹⁷⁰

It has been reported that vitamin B₁₂ in large doses (1,000 micrograms per week) is effective in relieving the neuritic pain which may

accompany arthritis,¹⁷¹ particularly when the symptoms are radicular in origin.¹⁷² It has also been recommended for the treatment of nutritional deficiency or anemia which may accompany arthritis.¹⁷³

Unconfirmed reports indicate the possibility of benefit from vitamin B₁₂ in other neuropathies. Parenteral administration of high doses over long periods has apparently resulted in amelioration or regression of some cases of neuroblastoma in children.¹⁷⁴ There have also been reports of prevention or alleviation of paralysis by vitamin B₁₂ in poliomyelitis.^{175,176}

LIVER DISORDERS

Numerous animal studies have indicated that vitamin B₁₂ has a protective effect on the liver during certain types of dietary deficiency during intake of toxic substances. Rats on an unsupplemented diet, for example, developed fatty livers which could be at least partially prevented by supplementation with methionine, choline or vitamin B₁₂.¹⁷⁷ Fatty livers were also prevented in alcohol intoxicated rats by daily doses of the vitamin.¹⁷⁸ Protective results were even more strikingly demonstrated when two groups of rats were fed a cysteine-free diet, with vitamin B₁₂ supplementation in only one group. The unsupplemented group developed, within a year, severe liver changes including focal necrosis, fatty metamorphosis, cirrhosis, duct hyperplasia or cysts. The group receiving vitamin B₁₂, on the other hand, showed evidence of liver disease only after two years, and even then the lesions were minimal.¹⁷⁹ That vitamin B₁₂ may affect liver regeneration after injury was indicated by another study in which rats deficient in riboflavin or vitamin B₁₂, or given supplements of these vitamins, were subjected to partial hepatectomy. A higher mortality rate and more limited regeneration occurred in the vitamin B₁₂ deficient than in the vitamin B₁₂ supplemented, riboflavin deficient, or control groups.¹⁸⁰

Encouraging results have been reported with the use of liver extract¹⁸¹ or vitamin B₁₂ in clinical studies of liver disease. The vitamin successfully reversed the fatal progress in all but the most advanced cases of infantile biliary cirrhosis.^{182, 183} In patients with fatty infiltration associated with diabetes or chronic alcoholism, the tender and excess volume of the liver receded rapidly following administration of vitamin B₁₂.¹⁸⁴ In a preliminary investigation, vitamin B₁₂ added to the conventional dietary treatment of a large group

patients with viral hepatitis, who were compared with two control groups. The vitamin B₁₂-treated patients regained normal appetite faster and liver size became normal more rapidly, serum bilirubin values returned to normal in 10 weeks, compared to 18 and 24 weeks in the other two groups, and the mean duration of illness was shortened by one week.¹⁸⁴ In a more recent study, 44 patients with viral hepatitis were given only the conventional diet, while a similar group was given, in addition, vitamin B₁₂ and folic acid. The results in the two groups closely paralleled those of the earlier study.¹⁸⁵ Though not all investigators report success in the treatment of viral hepatitis with vitamin B₁₂,¹⁸⁶ results such as those described suggest that vitamin B₁₂ may be a useful adjunct in therapy of hepatic disorders.

Studies in patients have indicated that the metabolism of vitamin B₁₂ is altered in both acute and chronic liver disease. Marked elevation of the serum vitamin B₁₂ levels has been found in several studies on patients with liver damage.¹⁸⁷⁻¹⁹¹ As the patients improve clinically, the serum vitamin B₁₂ concentrations tend to return toward the normal range, and conversely, when patients in hepatic coma become moribund the serum vitamin B₁₂ levels become more abnormal.¹⁹¹ In hepatic coma levels of from 30 to 40 times normal were found.¹⁸⁹ In viral hepatitis the serum concentrations have ranged as high as 20 times the usual values.¹⁹⁰ In alcoholic cirrhosis the elevation has been 3 to 8 times normal.¹⁸⁸ It has been suggested that the reason for the elevated serum levels is the release of the vitamin by damaged liver cells.¹⁹⁰⁻¹⁹¹ Lowered uptake by damaged liver cells, as demonstrated by studies with radioactive vitamin B₁₂,¹⁹² may also contribute to the elevated serum levels.

The binding of vitamin B₁₂ by the serum in liver disease, as well as the total quantity present, varies with the nature of the disorder. It has been found that patients with acute inflammation and necrosis have high levels of the free vitamin,¹⁹¹⁻¹⁹³ while patients with chronic liver involvement have high levels of the bound vitamin.¹⁹¹ It is believed that the increase in bound vitamin in such cases may be due to the production of abnormal amounts of binding substance by the damaged liver.¹⁹¹ It has been postulated that much of the vitamin found in the serum in such cases may be abnormal and thus physiologically inactive and that the body tissues might be relatively unsaturated in spite of the high serum levels. Thus, a deficiency of physiologically active vitamin B₁₂ might exist even in the presence of the high serum

levels This is of interest in view of the reports of benefit from vitamin B₁₂ therapy in liver disease ^{184,185}

Since the metabolism of vitamin B₁₂ is changed in liver disease, it has been suggested that such alterations might assist in evaluating the clinical status of patients ^{193 194} In a study of 3 normal subjects, and of 20 patients, 13 of whom had liver disorders, the normal subjects excreted between 24 and 40 micrograms of vitamin B₁₂ in eight hours following a 50 microgram intramuscular load dose, but none of the patients except some who were convalescing from liver disorders excreted more than 10 micrograms In view of these findings, the authors suggest that increased vitamin B₁₂ excretion after a load dose might indicate that the liver had recovered from damage and no longer needed increased amounts of vitamin B₁₂ for normal metabolic activity It is also suggested that the excretion following a load dose may aid in detecting the extent of liver damage and the progress of convalescence ¹⁹⁴ In line with this, it has been suggested ¹⁹³ that the changes in serum binding capacity which characterize some types of liver disease may also help in evaluating the clinical status or in differentiating various types of liver involvement In infectious hepatitis and in homologous serum jaundice, for example, binding capacities were found to be very low during the period of hyperbilirubinemia, but rose during convalescence In cirrhosis, however, the binding capacity decreased little or not at all Moreover, the total serum levels were generally lower in cirrhosis than in hepatitis The authors suggest that these differences might be employed in conjunction with the standard tests of liver function to aid in the differential diagnosis of these liver disorders

SKIN DISORDERS

Several investigators have reported that vitamin B₁₂ given parenterally in large doses, exerts a beneficial effect on some skin disorders

Lupus Erythematosus

Investigators have reported varying results with the use of vitamin B₁₂ in the treatment of lupus erythematosus In a preliminary study of 3 patients with chronic discoid and one with subacute disseminated lupus erythematosus, good response in clearing of the skin lesions was obtained with intramuscular injection of small doses, and further investigation with larger doses was recommended ¹⁹⁵ In another study

complete clearing of the skin lesions in 3 patients who had failed to respond to other therapy was reported following 12 semi weekly injections of 1,000 micrograms of vitamin B₁₂ ¹⁹⁶

In a study of 17 patients with chronic discoid lupus erythematosus, intramuscular injections of 1,000 micrograms three times weekly gave varying responses. In one patient the lesions cleared almost completely, 2 were much improved and 2 were slightly improved. In 9 patients there was no change in the lesions and in 3 the condition became worse, necessitating a change of treatment ¹⁹⁷

In another study it was reported that discoid as well as disseminated lesions responded even to small doses of vitamin B₁₂. Healing occurred without new pigmentation, scarring, or atrophy, but atrophy already present could not be reversed ¹⁹⁸

Seborrheic Dermatitis

In 16 of 36 patients with seborrheic dermatitis given supplemental treatment with intramuscular vitamin B₁₂, great improvement occurred, and moderate improvement was observed in 16 others. The few relapses which took place responded to further therapy. Other metabolic, endocrine or nutritional therapy was prescribed as needed and foci of infection were treated with sulfonamides or penicillin. The investigators believed the favorable results obtained with vitamin B₁₂ were based on the possible nutritional origin of this skin disorder ¹⁹⁹

Other Dermatoses

In a study of the use of intramuscular vitamin B₁₂ in various skin disorders, ²⁰⁰ substantial improvement was noted in 6 out of 10 patients with atopic dermatitis, in all of 6 with chronic contact dermatitis and in 9 out of 10 with chronic urticaria. In 2 patients with dermatitis herpetiformis, however, vitamin B₁₂ was ineffective.

Some patients with skin manifestations of drug reactions have benefited from the administration of vitamin B₁₂. Skin lesions and pruritus caused by therapeutic administration of gold salts have been alleviated or prevented by vitamin B₁₂. ²⁰¹ In a recent study of various dermatoses of allergic etiology it was reported that in all of 5 patients taking penicillin streptomycin injections and in all of 4 using iodine topically dermatitis healed after vitamin B₁₂ therapy. Eight of these patients were able to continue the offending medication. ⁹²

Several investigators have reported that skin lesions, as well as the neurologic symptoms, of patients with herpes zoster were improved by vitamin B₁₂ therapy¹⁵⁵⁻¹⁵⁷ It has been reported that vitamin B₁₂ administered parenterally caused a favorable result in 31 out of 35 patients with xanthelasma²⁰³

In a preliminary study of the treatment of patients with long standing psoriasis, large intramuscular injections of vitamin B₁₂ produced involution of lesions in 11, 75 to 80 per cent improvement in 10, gradual improvement in 6, temporary improvement in 5, and no improvement in 2 patients²⁰⁴ Some confirmation of these results is provided by a report on 17 resistant cases of psoriasis in which vitamin B₁₂ caused sustained improvement in 9, some improvement in 3, and no improvement in 5²⁰⁵

ENDOCRINE DISTURBANCES

Studies in animals have indicated that an increased vitamin intake is beneficial in certain hypohormonal or hyperhormonal states For example, supplements of vitamin B₁₂ enabled rats to withstand the effects of large doses of cortisone, thyroid, thiouracil, and diethylstilbestrol, as well as the stress of adrenalectomy and thyroparathyroidectomy

The reason for such beneficial effects is not completely understood It has been suggested, however, that the improved appetite and food utilization accompanying the increased vitamin intake may have been responsible, a hypothesis which is borne out by the fact that such effects occurred only when unlimited food was available²⁰⁶

It has been postulated that during hyperhormonal states, additional vitamins are needed to detoxify or eliminate the excess hormone In hormonal deficiency states, on the other hand, the increased vitamin intake might permit metabolic reactions to proceed at an improved rate in spite of the deficiency²⁰⁶

One interesting paradox has been noted in this connection It is commonly thought that the metabolic rate determines the requirements for the B vitamins Thus, in states characterized by a reduced metabolism, the need for these vitamins should be less Nevertheless, it has been noted that in hypothyroidism and diabetes mellitus, for example, beneficial effects have followed their administration²⁰⁶

Such observations have led to speculation as to whether or not vitamin B₁ plays a part in the functioning of the endocrine system

Adrenal Function

On the basis of observations made on patients with pernicious anemia it has been suggested²⁰⁷ that a deficiency of vitamin B₁₂ might lead to functional impairment of the adrenal cortex or the anterior lobe of the pituitary gland, either as a result of the general debilitating effect of the deficiency or because vitamin B₁₂ is actually necessary for the proper functioning of the glands. If vitamin B₁₂ is necessary for proper adrenal function it is possible that a metabolic antagonist to vitamin B₁₂ might induce adrenocortical insufficiency. Manifestations of adrenal insufficiency were reported to occur more often in pernicious anemia patients than in those with comparable degrees of anemia due to loss of blood.²⁰⁷ In a recent study, however,²⁰⁸ no evidence was found that adrenocortical function or steroid metabolism was impaired in 8 patients with pernicious anemia in relapse.

Thyroid Function

Anemia due to endocrine deficiency is most common in association with hypothyroidism. Anemia also accompanies insufficiency of the anterior pituitary and of the adrenal cortex. In these anemias, the bone marrow is somewhat hypoplastic. Although the anemia associated with severe hypothyroidism is usually hypochromic, macrocytic anemia has also been observed and these anemias clearly represent deficiencies of iron and vitamin B₁₂ superimposed on thyroid deficiency. Thyroid deficiency alone may limit the rate of hemopoiesis. The treatment of the mild anemia of endocrine disorders, however, is largely incidental to the management of the conditions themselves.

In humans it has been noted that patients suffering from pernicious anemia complicated by severe hypothyroidism may require higher dosages of thyroid extract when they are receiving vitamin B₁₂. In some such cases the dosage had to be doubled to effect a normal metabolic rate.²⁰⁹ Because of this observation it has been postulated that vitamin B₁₂ has antithyrototoxic effects and therefore might be a useful adjunct in the treatment of thyrotoxicosis.²⁰⁹ However, further investigation is necessary to test the validity of this idea.

Diabetic Retinopathy

The ocular complications of diabetes have a definite relationship to the duration of the disease and to its control. While the pathogenesis of these lesions is not yet understood, recent studies on vitamin

B₁₂ and diabetic retinopathy have thrown some light on the etiology of this complication.²¹⁰ Some association has been indicated between adrenocortical function, vitamin B₁₂, and diabetic retinopathy. Decreased adrenocortical function, as measured by responsiveness to corticotropin, was found in diabetic patients without retinopathy, while those with retinopathy had normal or increased function.²¹¹ A relationship between retinopathy and Kimmelstiel Wilson lesions has been indicated by studies which showed that at autopsy 80 to 90 per cent of diabetic patients with nephropathy also had retinopathy, and about 50 per cent of those with symptoms of retinopathy proved at autopsy to have kidney lesions.²¹²

Since study in humans showed that the urinary excretion of vitamin B₁₂ given intramuscularly was much less from diabetic patients than it was from normal subjects,²¹³ it was thought that a relationship might exist between the amount of vitamin B₁₂ excreted in the urine of diabetic patients and the presence or absence of retinopathy. To test this hypothesis, 50 micrograms of vitamin B₁₂ was given to 35 diabetic patients, 22 with and 13 without retinopathy, and to 6 healthy persons. Diabetic patients with retinopathy excreted an average of approximately 19 micrograms of vitamin B₁₂ in eight hours, those without retinopathy an average of only 4.2 micrograms, and normal control subjects an average of 9.6 micrograms. These differences between patients with and without retinopathy were statistically significant, but the authors were unable to explain their meaning, furthermore no correlation was found between the severity of the retinopathy and the vitamin B₁₂ excretion.²¹³ Depression of adrenocortical function in diabetic patients with retinopathy by administration of *testosterone* caused a decrease in their excretion of vitamin B₁₂.¹⁴⁵ As a result of such studies it has been suggested that excessive adrenocortical function and a deficiency of vitamin B₁₂ may be involved in Kimmelstiel-Wilson and retinopathic lesions.¹⁴⁵

Of interest in connection with the relationship of vitamin B₁₂ to complications of diabetes, such as diabetic retinopathy, are studies in animals indicating that vitamin B₁₂ is involved in carbohydrate metabolism. In rats, vitamin B₁₂ deficiency causes hyperglycemia which is corrected by administration of the vitamin. Moreover, cortisone induced hyperglycemia is accompanied by destruction of vitamin B₁₂ binding substances in muscle, and an elevated vitamin B₁₂ serum level. Whether this has any connection with the apparent adrenal hyper

activity and increased excretion of the vitamin found in patients with retinopathy has not yet been determined ²¹¹

CLINICAL INDICATIONS FOR DIETARY SUPPLEMENTATION

Nutritional disorders may result from any of the following factors poor dietary intake, poor absorption, metabolic disturbances leading to nausea, vomiting diarrhea, and increased metabolism, and stress conditions such as severe or prolonged infection, injury, or surgery, particularly surgery of the gastrointestinal tract. It is well established that vitamin supplementation is beneficial in such conditions and that vitamin B₁₂ plays an important role in such supplementation ²¹⁵

Retarded Growth

Since large scale depletion studies are usually not conducted in human beings, the most significant findings are evident in infants or children who are deprived of animal protein for prolonged periods because of inadequate diet or who are unable to assimilate vitamin B₁₂ because of illness

Early controlled studies on oral administration of vitamin B₁₂ to undernourished children with growth failure showed that statistically significant growth increases occurred in the treated children ^{216 217}. Later studies on a large group of underweight Italian children who were eating diets low in animal protein also showed that those receiving supplements of vitamin B₁₂ gained more weight than the controls ²¹⁸. These findings are supported by other controlled studies ²¹⁹⁻²²¹. Uncontrolled studies in malnourished children have also indicated beneficial effects, but results in these groups are, of course, difficult to evaluate ^{222 223}. Although these studies in general show a growth effect in malnourished children with retarded growth, no beneficial effect was demonstrated in other controlled studies ²²⁴⁻²²⁷.

When normally nourished but chronically ill children received vitamin supplements including vitamin B₁₂, their rate of growth was significantly greater than that of the controls ^{218 219}. Beneficial effects including increases in weight and improved blood protein levels, were also observed in acutely ill children with respiratory and cardiovascular disease following administration of vitamin B₁₂ and liver extract ²³⁰. These results on sick children indicate that the need for vitamin B₁₂, like the need for other vitamins, may be increased during illness.

Because many studies indicate increased growth in children fol

lowing vitamin B₁₂ therapy, it has been suggested that the vitamin may participate in some fundamental metabolic growth regulating mechanism.²²¹ It must be emphasized, however, that most of these studies showed a beneficial effect of vitamin B₁₂ on growth only in poorly nourished children. Further extensive investigation is necessary before definite conclusions can be reached.

Vitamin B₁₂ has produced beneficial effects on anorexia in children. Increased appetite has been reported in two groups of healthy, but anorectic, children given supplemental vitamin B₁₂.²³¹⁻²³² In a study of underweight children, 114 of 145 showed a marked increase in appetite, which was reflected in an increase in rate of weight gain.²³³

Pregnancy

Several studies indicate that an increased need for vitamin B₁₂ may exist during pregnancy, possibly because large amounts of the vitamin are needed for growth and blood formation in the fetus. A comparative study in pregnant and nonpregnant women indicates that the absorption of vitamin B₁₂ from the intestine may be increased during pregnancy.²³⁴ That this increased absorption may be a mechanism of compensation for the depletion of the mother's vitamin B₁₂ stores is suggested by several studies which show that maternal vitamin B₁₂ serum levels progressively decrease during pregnancy.¹³³⁻²³⁴⁻²³⁶ Assays of the serum obtained from 502 women at various stages of pregnancy showed an unmistakable tendency for a decrease of the vitamin B₁₂ serum level with the progress of pregnancy. Even during the first trimester the average value was below that previously established for normal males and nonpregnant females.²³⁵ A study of pregnant women during the second and third trimester indicates that the serum levels of vitamin B₁₂ may decrease to levels comparable to those found in pernicious anemia.²³⁶ A study made during the third trimester showed that normal or low vitamin B₁₂ serum levels progressively decreased until after delivery, when they rose again.¹³³

In another study vitamin B₁₂ serum levels were determined in 25 mothers and infants at the time of delivery.²³⁷ It was found that the vitamin B₁₂ serum level of the maternal blood was considerably lower than that of nonpregnant women of comparable age. It was also lower than the vitamin B₁₂ level in the fetal cord blood. These results were confirmed by a study of 96 paired serum samples taken from mothers and infants at delivery which showed that the average serum vitamin

B₁₂ concentration in the infant was about twice that in the mother. In some cases the infant's vitamin B₁₂ serum concentration was six times that of the mother.²³⁹ These studies suggest the possible need for supplementary vitamin B₁₂ during pregnancy even in the absence of frank macrocytic anemia.

Several animal studies provide evidence that vitamin B₁₂ is necessary for the proper growth and development of the fetus. Deprivation of vitamin B₁₂ in female rats, for example, throughout the period of mating and gestation, resulted in weak, defective, underweight offspring. Developmental defects were noted in the heart and kidneys, and in the spleen the reticular cells were large, densely chromatic and pyknotic. Vascular blocks with passive congestion, especially in the liver, were observed.²³⁹ In a study on a different strain of rats, the congenital anomalies resulting from vitamin B₁₂ deficiency took the form of hydrocephalus or defects in eye and bone development.²⁴⁰

While it is difficult to apply the results of these studies to human beings, they suggest the need for further studies on the effect of vitamin B₁₂ on the fetus, particularly since it has been established that maternal vitamin B₁₂ serum levels are frequently lowered in pregnancy.

Clinical Indications in Geriatric Patients

Vitamin deficiencies may occur in the elderly patient when, because of poor dentition, poverty, indifference or chronic gastritis, an inadequate diet is taken.²⁴¹ Even when their diet is normal, however, elderly people frequently have special nutritional problems because of the physiologic changes which accompany aging.²⁴¹ Improvement in the status of geriatric patients following the administration of vitamin B₁₂ alone or in combination with other vitamins has been reported.^{242, 243}

One physiologic change observed in older persons is a decreased capacity of the gastric juice to bind vitamin B₁₂.^{244, 245} Achlorhydria and hypochlorhydria are also found in the aged and it is probable that vitamin B₁₂ deficiency due to poor absorption may occur with some frequency in the aged.²⁴⁶ This is consistent with the observation that older people absorb orally administered vitamin B₁₂ less readily than do younger persons. In a recent study, a group of persons between 20 and 40 years of age all showed a significant increase in the serum vitamin B₁₂ level after a large oral dose, while only 6 out of 18 in the group between 60 and 90 years of age showed a comparable increase.²⁴⁴

Decreased absorption of orally administered vitamin B₁₂ in older

persons results in lower blood levels of the vitamin²⁴⁷ and probably in lower tissue levels.²⁴⁴ Assays of serum samples from randomly selected populations of different age groups show that the vitamin B₁₂ serum content decreases with advancing age.²⁴⁸ In a group of old individuals on a satisfactory diet containing adequate vitamin B₁, the mean serum vitamin B₁₂ level was significantly lower than the mean serum level found in a group of younger individuals on a normal diet.²⁴⁸ In a study of five groups of apparently healthy subjects, composed of a total of 528 individuals ranging in age from 12 to 94, it was found that the mean serum vitamin B₁₂ levels in each group showed an almost uniform gradual decline with advancing age. Low serum vitamin B₁₂ levels were found more frequently in persons over 50 years old than in younger persons.²⁴⁹ That the tissue levels as well as the blood levels of vitamin B₁₂ in older persons are decreased is indicated by studies which show that more injected vitamin B₁₂ is retained by older than by younger persons, indicating less vitamin B₁₂ saturation in the tissues of the former.²⁴⁴

In a discussion of the nutritional rehabilitation of the elderly patient undergoing surgery, one investigator has noted that subclinical vitamin deficiencies are common among this group because of anorexia, lack of balanced diet, infection, intestinal disorders and long illnesses. Therefore vitamin B₁₂ was suggested as an adjunct to therapy in the elderly patient undergoing operation or any other severe stress.²⁵⁰

Stress

The findings of several investigators have suggested that the need for vitamin B₁₂ during periods of stress may be increased beyond the amount supplied by the diet. For example, increased vigor and sense of well-being have frequently been observed in patients with a variety of illnesses who have been treated with vitamin B₁₂, even when no direct effect upon the disease was apparent.^{197 251-253} In children, the most striking growth increments have followed vitamin B₁₂ therapy in those who were undersized because of previous illness or inadequate nutrition.^{216 217 228 230}

In line with these observations, the suggestion has been made that patients with illness or trauma, as well as those depleted nutritionally, be given supplemental vitamin B₁₂.²¹⁵ It has been pointed out in this connection that during severe illness, following burns, after radiation injury, or in certain pathologic states, hemopoiesis may be depressed

and enzyme activity may be disturbed. Since vitamin B₁₂ is involved in normal hemopoiesis and probably in some enzymatic processes, its regular administration, along with folic acid, has been recommended as part of the dietary supplements used in hospitals when the diet intake is limited.²⁵⁴

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- 249 Gaffney G W Horonick A, Okuda K Meier P Chow H F and Shock N W Vitamin B₁₂ serum concentrations in 528 apparently healthy human subjects of ages 12 to 94 *J Gerontol* 11 32-38, Jan 1957
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Part 3

Selected Annotated Bibliography

INTRODUCTION

This annotated bibliography comprises abstracts of selected material appearing in the medical and related scientific literature since July 1953. In choosing many of the abstracts an attempt has been made to present recent reports on the established clinical uses of vitamin B₁₂ and also to draw attention to the various diseases and disorders in which vitamin B₁₂ has been reported to have therapeutic or nutritional application. This bibliography is designed to supplement the comprehensive collection of abstracts and reference data presented in the Merck Service Bulletin on Vitamin B₁₂, Part 2, Annotated Bibliography, published in 1954.

BLOOD AND LYMPHATIC ANEMIAS

MACROCYTIC ANEMIAS GENERAL

- 1 Nelson N A and Bishop, R C. Nonaddisonian megaloblastic anemia Clin Research Proc. 4 234 Nov 1956 (in Soc. Proc.)

Pernicious anemia either in relapse or remission, may now be diagnosed by demonstrating the absence of intrinsic factor activity by means of radioactive vitamin B₁₂ studies. Such studies may also be of help in the diagnosis of pernicious anemia in patients with neurologic disease resembling subacute combined degeneration without anemia or in those who have received inadequate therapy.

Recently radioactive vitamin B₁₂ was used to study 2 patients with macrocytic megaloblastic anemia and histamine fast achlorhydria. One had a history of dietary insufficiency and purpuric skin lesions suggesting scurvy. After the Schilling test effectively excluded the diagnosis of pernicious anemia, he improved without therapy other than diet. The other patient was an epileptic on Dilantin. An adequate hematologic response occurred in this patient during a trial with investigational material. The Schilling test performed on 2 occasions however demonstrated normal urinary excretion of radioactive vitamin B₁₂, again eliminating the diagnosis of addisonian pernicious anemia and the need for continued vitamin B₁₂ therapy.

In a third patient with nutritional megaloblastic anemia, free hydrochloric acid was present in the gastric juice. A radioactive vitamin B₁₂ excretion test confirmed the presence of intrinsic factor activity.

Wayne County Gen. and Hospital
Elaine Mich

- 2 Mollin D L and Ross G I M. Serum vitamin B₁₂ concentrations of patients with megaloblastic anemia after treatment with vitamin B₁₂, folic acid or folinic acid. Brit M J 2 640-645, Sept 19 1953.

Single intramuscular injections of 20 to 1 000 mcg of vitamin B₁₂ were given to 33 patients with pernicious anemia in relapse. Concentrations of vitamin B₁₂ in serum (as shown by Euglena gracilis assay) rose from low pretreatment levels and remained within normal range for lengths of time somewhat dependent on the size of the dose: e.g. 6 to more than 18 days after 20 mcg, 11 to at least 35 days after 80 mcg, 34 to at least 58 days after 1 000 mcg. Although concentrations were within the normal range for these periods, the mean of the concentrations of all patients in each group fell below the average found in normal subjects within a few days of injection.

Serum vitamin B₁₂ levels were maintained within the normal range in most patients who received 40 mcg of the vitamin every 10 days or 160 mcg every 21 days. The authors state that these doses appear to be the minimum requirement for patients in relapse but they recommend that large doses (up to 5 mg) be given in the first week or two.

When serum vitamin B₁₂ concentrations were normal, hemopoiesis was normal. When concentrations were below normal, megaloblasts reappeared in the marrow. In 12 patients with megaloblastic anemia of sprue or pregnancy, serum concentrations of vitamin B₁₂ were normal. Treatment of these patients with vitamin B₁₂ was ineffective and treatment with folic or folinic acid, which was effective, did not affect serum levels of vitamin B₁₂.

Postgraduate Medical School of London
London, England

- 3 Nieweg H O Faber, J G de Vries J A and Kroese, W F S The relationship of vitamin B₁₂ and folic acid in megaloblastic anemias, *J Lab & Clin Med* 44 118 132, July 1954

Patients with megaloblastic anemia who have neurologic disorders respond to vitamin B₁₂ therapy. Such patients have low serum levels of this vitamin and normal or low folic acid activity of whole blood. Patients with megaloblastic anemia who respond only to folic acid have normal or high serum levels of vitamin B₁₂ and low whole blood folic acid activity.

In 7 untreated cases of pernicious anemia intramuscular injections of vitamin B₁₂ caused a decrease in the folic acid activity of the blood. The value rose again after 72 hours. A review of theories and data concerning folic acid metabolism in pernicious anemia led to the conclusions that vitamin B₁₂ administration causes an increased utilization of folic acid in the bone marrow, has some influence on its storage in the liver and possibly causes an increased intake of folic acid.

The function of folic acid and vitamin B₁₂ in nucleic acid synthesis is discussed. The authors suggest that vitamin B₁₂ is necessary for the production of both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) while folic acid participates only in the synthesis of the latter. Since RNA takes part in protein synthesis and DNA is a constituent of the chromosomes in the nuclei of cells, deficiency of vitamin B₁₂ produces both lesions of the nervous system (RNA) and hematologic disorders (DNA) while folic acid deficiency causes only the latter. The authors emphasize that this is theoretical speculation only.

*State University of Groningen
Groningen, Netherlands*

- 4 Lipetz S Prostatic carcinoma and macrocytic anemia. *Lancet* 2 498 Sept 4, 1954 (Letter to Editor)

Vitamin B₁₂ was 81 year
old man for macrocytic de

veloped about two years after the of the prostatic carcinoma. Both and urinary symptoms disappeared automatically soon after vitamin B₁₂ therapy was begun.

The case is presented as relevant to the hypothesis that there are possible connections between cancer and other metabolic diseases because (1) Pernicious anemia has its associated neuropathy and an association with gastric carcinoma. There is reason to suppose that the same unknown factors interfering with haemoglobin synthesis and red-cell formation in the presence of certain plasma factors. (3) Animal experiment has suggested that vitamin B₁₂ might be effective in the treatment of some cases of malignant disease and it has recently been reported that several cases of neuroblastoma have shown remarkable and long remission after treatment with vitamin B₁₂.

Edinburgh, Scotland

- 5 Mueller J F and Will J J The relationship of folic acid, vitamin B₁₂ and ascorbic acid in patients with megaloblastic anemia. *Am J Clin Nutrition* 3 30-44, Jan-Feb 1955

The authors discuss the chemical relationships of vitamin B₁₂ and folic acid in bringing about the maturation of pernicious anemia megaloblasts. It is proposed that vitamin B₁₂ and folic acid act as catalysts in a reaction leading to the formation of nucleic acid and that ascorbic acid affects folic acid metabolism. Does vitamin B₁₂, a deficiency of vitamin B₁₂ will result in a conditioned deficiency of folic acid. Clinical experiments formed to test this hypothesis are described.

The authors conclude that folic

In pernicious anemia folic acid therapy temporarily corrects the defect in nucleoprotein metabolism by mass action. Eventually a greater deficiency of vitamin B₁₂ occurs, resulting in hematologic relapse and progressive neurologic disease.

Pernicious anemia of pregnancy and vitamin B₁₂ refractory megaloblastic anemia also occur because of defects in nucleoprotein metabolism. These anemias are caused by deficiencies of folic acid coenzyme. Ascorbic acid metabolism also may be altered in patients with megaloblastic anemias since vitamin C is capable of producing reticulocytosis and improvement in some of these patients.

*University of Cincinnati
College of Medicine
Cincinnati, Ohio*

- 6 Vilter R. W. Treatment of macrocytic anemias. *A M A Arch Int Med* 95:482-492 March 1955 (in Treatment in Internal Medicine)

The author reviews the literature on macrocytic anemias and discusses their differential diagnosis. For therapy in uncomplicated pernicious anemia he compares the parenteral use of vitamin B₁₂ and refined liver extract and states that vitamin B₁₂ is cheaper and causes so few reactions that it is the preferred agent. There is no evidence that liver extract will induce any better response than vitamin B₁₂, nor is there any evidence that folic acid, ascorbic acid, or iron is needed in the average case. He does not favor the oral vitamin B₁₂-intrinsic factor preparation. Specific recommendations for vitamin B₁₂ dosage are given. Iron is recommended when hypochromia is present, folic acid is recommended when a dietary inadequacy is persistent and anemia reappears in spite of vitamin B₁₂ therapy. Vitamin B₁₂ is recommended in nutritional macrocytic anemia and sprue although supplementary folic acid may be required in the latter disease.

*University of Cincinnati
College of Medicine
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- 7 Unglaub W. G. and Goldsmith G. A. Oral vitamin B₁₂ in the treatment of macrocytic anemias. *South M J* 48:261-269 March 1955.

Large oral doses of vitamin B₁₂ were given to 11 patients with pernicious anemia and to 3 patients with nutritional macrocytic anemia. Dosages varied from 3,000 mcg every four weeks to 1,000 mcg weekly. One patient received 3,000 mcg weekly. Maintenance therapy continued for from 4 to 28 months. Of the patients with pernicious anemia, 5 had received no previous therapy, the other 6 had previously received various types of therapy.

Satisfactory hematologic response was seen in all patients. Serum vitamin B₁₂ in 9 increased from pretreatment levels of 0.02 to 0.05 millimicrograms per cc to levels of 0.10 to 0.68 millimicrograms per cc. In 3 pernicious anemia patients with subacute combined sclerosis of the spinal cord, marked improvement followed oral vitamin B₁₂ therapy. Of the patients with nutritional macrocytic anemia, 2 relapsed after vitamin B₁₂ therapy was withdrawn in spite of diets considered nutritionally adequate. One had a decrease in serum vitamin B₁₂ levels paralleling the fall in the blood count.

*Tulane University School of Medicine and
Charity Hospital of Louisiana
New Orleans, La.*

- 8 Watkins C. H. The treatment of anemia. *Minnesota Med* 38:327-330 May 1955.

Effective doses of liver extract or vitamin B₁₂ are important in the treatment of pernicious anemia. Initial doses of 45 units liver extract or 60 mcg vitamin B₁₂ are given intramuscularly daily for three days, followed by 15 units liver extract or 30 mcg vitamin B₁₂ once a week until the blood picture becomes normal. Maintenance doses are continued for life. Responses to liver extract and vitamin B₁₂ are essentially the same. Vitamin B₁₂ has

- 3 Nieweg H O, Faber, J G de Vries, J A and Kroese W F S The relationship of vitamin B₁₂ and folic acid in megaloblastic anemias J Lab & Clin Med 44 118-132 July 1954

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Vitamin B₁₂ was given to an 81 year old man for macrocytic anemia that de-

veloped about two years after the onset of the prostatic carcinoma. Both anemia and urinary symptoms disappeared dramatically soon after vitamin B₁₂ therapy was begun.

The case is presented as relevant to the hypothesis that there are possible links between cancer and other metabolic processes because (1) Pernicious anemia has its associated neuropathy and a direct association with gastric carcinoma (2) There is reason to suppose that there are some unknown factors interfering with haemoglobin synthesis and red-cell formation in the presence of certain neoplasms (3) Animal experiment has suggested that vitamin B₁₂ might be effective in the treatment of some cases of malignant disease and it has recently been reported that several cases of neuroblastoma have shown remarkable and lengthy regression after treatment with vitamin B₁₂.

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The authors conclude that folic acid and vitamin B₁₂ deficiency states are associated with defects in nucleoprotein metabolism characterized biochemically by increased ratios of ribonucleic acid to deoxyribonucleic acid and uracil to thymine. These biochemical changes are correlated with the degree of megaloblastosis.

In pernicious anemia folic acid therapy temporarily corrects the defect in nucleoprotein metabolism by mass action. Eventually a greater deficiency of vitamin B₁₂ occurs resulting in hematologic relapse and progressive neurologic disease.

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Tulane University School of Medicine and
Charity Hospital of Louisiana
New Orleans, La.

8 Watkins C. H. The treatment of pernicious anemia. Minnesota Med. 38:1-5 May 1955

Effective doses of liver extract and vitamin B₁₂ are important in the treatment of pernicious anemia. Initial doses of 60 units of liver extract or 60 mcg. of vitamin B₁₂ are given intramuscularly daily for 7 days followed by 15 units of liver extract or 30 mcg. vitamin B₁₂ once a week. When the blood picture becomes normal maintenance doses are continued. Responses to liver extract and vitamin B₁₂ are essentially the same.

however an advantage over liver extract in that it is a purified crystalline substance of constant potency which may be used in patients sensitive to liver extract it produces a minimal local reaction Folic acid citrovorum factor, liver extract combined with iron and hydrochloric acid although not contraindicated are not preferred methods of therapy

Other types of megaloblastic anemia are often difficult to treat If no response occurs after two or three weeks of vitamin B₁₂ therapy folic acid administration should be tried then citrovorum factor Occasionally a rare macrocytic anemia with a relatively aplastic bone marrow responds to cortisone then to folic acid or vitamin B₁₂ In refractory anemias such treatment might be tried

*Mayo Foundation and Mayo Clinic
Rochester Minn*

- 9 Girdwood, R II The megaloblastic anemias Their investigation and classification, *Quart J Med* 25 87-119 1956 (abstr *Blood* 11 1053, Nov 1956)

This account seeks to classify the megaloblastic anemias largely from estimation of folic acid absorption and of the serum vitamin B₁₂ level Pernicious anemia may be diagnosed by the serum vitamin B₁₂ estimation before megaloblastic anemia develops The folic acid absorption test showed malabsorption to be present in 22 cases of idiopathic steatorrhea investigated whether or not megaloblastic anemia was present

This malabsorption was not present in other forms of megaloblastic anemia steatorrhea and megaloblastic anemia after gastrectomy or gastroenterostomy were associated with vitamin B₁₂ deficiency but not with malabsorption of folic acid Megaloblastic anemia of pregnancy was not associated with vitamin B₁₂ deficiency or with folic acid malabsorption but there are several types of megaloblastic anemia that may occur in

pregnancy When a patient with vitamin B₁₂ deficiency is treated with folic acid for a long period megaloblastosis may recur

*University of Edinburgh
Edinburgh Scotland*

PERNICIOUS ANEMIA

PERNICIOUS ANEMIA DIAGNOSTIC STUDIES

- 10 Graham R M and Rheault M II Characteristic cellular changes in epithelial cells in pernicious anemia *J Lab & Clin Med.* 43 235-245, Feb 1954

Specific cellular changes occurring in the epithelial cells of 41 pernicious anemia patients were studied Twenty patients were in relapse and 21 were in therapeutic remission All patients had severe macrocytic anemia before treatment and all showed a therapeutic response to vitamin B₁₂ or liver therapy All had histamine fast achlorhydria Definite changes in the squamous epithelial cells the columnar epithelial cells and the histiocytes found in the gastric secretions of patients with untreated pernicious anemia and patients in relapse were observed During treatment these changes markedly decreased and were almost completely absent in patients in clinical remission following vitamin B₁₂ or liver therapy

Though this study was mainly concerned with the cellular picture of the gastric secretion specific abnormal cell forms were also found in the sputum in smears from the oral cavity and in the vaginal smear The suggestion is made that the extrinsic factor (vitamin B₁₂) may be essential for the normal maturation of epithelial cells just as it is for the normal maturation of the red blood cells

*Vincent Memorial Hospital and
Massachusetts General Hospital
Boston Mass*

- 11 Weaver J A and Neill D W Amino-aciduria in pernicious anemia and subacute combined degeneration of the cord *Lancet* 2 1212 1213, June 17 1954

Five patients with pernicious anemia showed abnormal excretion of taurine lysine cystine and leucine which reverted toward normal after treatment with vitamin B₁₂. Five patients with other types of anemia showed no abnormal amino-acid pattern in the urine. The damage caused or the area affected by the lack of vitamin B₁₂ in patients with pernicious anemia has not been determined.

One case of subacute combined degeneration of the cord is discussed in detail. Before treatment there was excessive excretion of taurine glycine alanine β -amino-iso-butyric acid and glutamic acid. Vitamin B₁₂ 100 mcg daily was given intramuscularly for 14 days followed by 100 mcg twice weekly. After this treatment the excessive taurine excretion ceased.

The possible causes of amino-aciduria in pernicious anemia are discussed and it is suggested that because vitamin B₁₂ is involved in the transmethylation of certain amino acids a failure of this action in pernicious anemia may be the basis of development of megaloblastic bone marrow. It is stated that further study of the amino-aciduria of untreated pernicious anemia may help determine the basic action of vitamin B₁₂.

The authors suggest that the association of achylia gastrica and increased taurine excretion in the urine may permit early diagnosis of subacute combined degeneration of the cord.

*Royal Victoria Hospital and
Queen's University
Belfast, North Ireland*

- 12 Conley C L Pitfalls in the diagnosis of pernicious anemia *Gen Practitioner* 11 59-61, Jan 1955

When a patient with symptoms of pernicious anemia responds dramatically to the parenteral administration of vitamin B₁₂, convincing support for this diagnosis

is provided. When folic acid alone is administered to such a patient the hematologic disorders may clear completely while neurologic degeneration continues. Thus in doubtful cases a thorough diagnosis must be made before any folic acid is administered. The author states that deficiency of folic acid is extremely rare in the United States and that its indiscriminate use is not justified in view of the difficulties it causes in the diagnosis of other diseases.

Preparations for oral use that combine vitamin B₁₂ and the intrinsic factor are not as effective as vitamin B₁₂ injected parenterally and should be considered experimental. An injection of 50 mcg of vitamin B₁₂ given once each six weeks constitutes good maintenance therapy. When a patient on this maintenance dose has a relapse the presence of another disease (for example carcinoma of the stomach with hemorrhage into the gastrointestinal tract and subsequent anemia) must be considered.

*Johns Hopkins University and Hospital
Baltimore Md*

- 13 Reisner D J and Ellsworth R M Coexistent idiopathic hypoparathyroidism and pernicious anemia in a young girl: case report *Ann Int Med* 43 1116-1124 Nov 1955

In a child with hypoparathyroidism and pernicious anemia correct diagnosis was established by the urinary phosphorus response to intravenous parathyroid hormone and by a diagnostic trial of daily oral doses of 5 mcg of vitamin B₁₂. The latter produced no reticulocyte response in eight days but reticulocyte response did occur when intrinsic factor was given with oral vitamin B₁₂. Anemia improved on a regimen of 50 mcg of vitamin B₁₂ given intramuscularly twice a week. The patient is being adequately maintained on a monthly intramuscular dose of 30 mcg of vitamin B₁₂ together with dihydroxy chysterol calcium gluconate and viosterol.

*St Luke's Hospital
New York N Y*

- 14 Rabiner, S F, Lichtman, H C, Messite, J, Watson, R J, Ginsberg V, Ellenbogen, L and Williams, W L. The urinary excretion test in the diagnosis of addisonian pernicious anemia, *Ann Int Med* 44 437-445, March 1956

The urinary excretion test with Co⁶⁰ vitamin B₁₂ was evaluated as a diagnostic procedure in pernicious anemia patients in hematologic remission. This test was also done in 24 control subjects in 36 patients with pernicious anemia and in 33 pernicious anemia patients who were given intrinsic factor. The procedure was to collect daily urinary output, the first day's specimen serving as control. On the second day 2 mcg of Co⁶⁰ vitamin B₁₂ was given orally before breakfast and simultaneously 1 000 mcg of nonradioactive vitamin B₁₂ was given intramuscularly (flushing dose). On the third day 1 000 mcg of vitamin B₁₂ was injected. On the fourth day intrinsic factor was given orally to 33 of the patients in addition to another intramuscular dose of 1,000 mcg of vitamin B₁₂. The results are shown in a table.

It is diagnostically significant that there is no overlap between the control group and the pernicious anemia patients not receiving intrinsic factor. Thus pernicious anemia may be definitely diagnosed or ruled out. In 26 of the 33 patients given intrinsic factor the percentage of urinary excreted radioactive vitamin B₁₂ increased to within the normal range.

*State University of New York College of Medicine
at New York City and
Kings County Hospital
Brooklyn N Y*

*Lederle Laboratories
Pearl River N Y*

- 15 Adams J F. Glossitis and the pre anaemic stage of pernicious anaemia. Diagnosis by simple methods. *Lancet* 1 1120-1121, June 1 1957

Diagnosis of the pre anaemic stage of pernicious anemia may be established by gastric aspiration bone marrow biopsy

and response to vitamin B₁₂ therapy. Suspicion is first aroused by symptoms such as glossitis or neuropathy.

The author presents histories of 3 patients with glossitis not accompanied by anemia. All 3 had histamine fast achlorhydria and a sternal marrow that showed hyperplastic megaloblastic erythropoiesis. Microbiologic assay revealed serum vitamin B₁₂ levels of 40, 45 and 120 micromicrograms per cc (normal mean 360 micromicrograms). Parenteral vitamin B₁₂ (dosage not stated) abolished the glossitis but did not alter the blood levels in the first patient.

Only one other comparable case has been reported in the literature but the syndrome may not be rare for the cases reported here constitute half the cases of glossitis without anemia seen in a year in a general medical unit. They demonstrate clearly that the diagnosis of pernicious anemia can be established in the pre anaemic stage.

*Western Infirmary
Glasgow Scotland*

- 16 Rubin C E, Phelps, P, Taniguchi, L and Fein, B. The pathogenesis of the precancerous gastric lesion in pernicious anemia—a new theory, *Clin Research Proc* 4 199-200, Sept 1956

The authors state that deficient intestinal absorption of Co⁶⁰ vitamin B₁₂ offers confirmation of pre-anaemic pernicious anemia sometimes suggested by atypical gastric cells. The cytologic study of a gastric polyp led to the discovery of a pre-anaemic state in an achlorhydric patient. Co⁶⁰ vitamin B₁₂ studies established the diagnosis in spite of normal neurologic and hematologic findings.

The authors conclude "The hereditary nature of PA [pernicious anemia] is established. Some believe that gastric atrophy causes the characteristically defective B₁₂ absorption. It is possible that the only inherited trait is a diminished ability to manufacture intrinsic factor. Thus gastric mucosal disease may be the result of B₁₂ deficiency rather than its

cause. Similarly the increased incidence of gastric polyp and carcinoma in PA may well be related to protracted B₁₂ deficiency.

Seattle Wash

- 17 Krstensen H P O and Olesen A S Early diagnosis of pernicious anemia with report of case Ugeskr f laeger 118 1228-1231, Oct III 1956 (abstr J A M A. 163 494 Feb 9 1957)

A case is described in which moderately grave achyllic iron deficiency anemia was accompanied by incipient pernicious anemia. Treatment with iron fully normalized the hemoglobin percentage but the vitamin B₁₂ content of the plasma was reduced. Subsequent bone marrow examinations revealed distinct megaloblastic changes which disappeared after parenteral treatment with vitamin B₁₂. Recognition of megaloblastic changes in the bone marrow in early pernicious anemia may be difficult. If such changes are suspected in patients with signs of iron deficiency the plasma vitamin B₁₂ level should be determined and examination of the bone marrow repeated after adequate treatment with iron.

- 18 Hanlon D G Dodge H W Jr Siekert R G and Bull F E Tumors of the spinal cord. Occurrence in patients with pernicious anemia and subacute combined sclerosis J A M A 162 707 709 Oct 20 1956

Vitamin B₁₂ was given parenterally to 11 patients with pernicious anemia and dorso-lateral spinal degeneration. Response was excellent but despite adequate maintenance therapy neurologic symptoms recurred in 1 patient after 11 months and in the other after 6 years. When the symptoms failed to respond to intensified vitamin B₁₂ therapy some cause of the neurologic symptoms other than pernicious anemia was sought. It was found that both patients had tumors (meningioma and neurofibroma) of the spinal cord as well as pernicious anemia. Prior

to removal of the tumors the diagnosis of pernicious anemia in both patients was confirmed by the oral absorption test with radioactive vitamin B₁₂.

Mayo Clinic and Mayo Foundation
Rochester Minn

PERNICIOUS ANEMIA TREATMENT

- 19 Cameron D G, Townsend M R and English A Pernicious anaemia II Maintenance treatment with crystalline vitamin B₁₂ Canad M A J 70 398-400 April 1954

Twenty six patients with pernicious anemia who had been satisfactorily maintained by intramuscular injection of 30 U S P units of concentrated liver extract weekly and 2 others who had been treated with vitamin B₁₂ were satisfactorily maintained on weekly injections of 30 mcg of vitamin B₁₂. The period of observation covered three to four years in 24 cases and over a year in 2. The results were comparable to those in 22 similar patients whose treatment was continued on liver extract.

McGill University Clinic of
The Montreal General Hospital
Montreal Canada

- 20 Schwartz S O, Friedman I A and Gant, H L Long term evaluation of vitamin B₁₂ in treatment of pernicious anemia. I Incidental report on use of combined oral therapy with vitamin B₁₂ and folic acid J A M A 157 229 231 Jan 15 1955

This long term evaluation of 32 patients with pernicious anemia treated with parenteral vitamin B₁₂ and 34 patients treated with liver extract covers a period of at least four years. The dosage of vitamin B₁₂ was 30 mcg every four weeks; the dosage of liver extract was 30 units each month.

In 4 patients neurologic relapse occurred after 4, 6, 13 and 28 months of vitamin B₁₂ therapy. Of these patients 3 improved when vitamin B₁₂ dosage was

increased another, whose relapse was severe improved after two months of weekly liver injections. None of the patients had hematologic relapses while receiving vitamin B₁₂.

Among the 34 patients treated with liver extract 3 had slight recurrence of paresthesias after 29, 30 and 20 months but all these patients responded favorably to increased doses of liver extract.

Oral treatment of pernicious anemia with 25 mcg of vitamin B₁₂ and 1.67 mg of folic acid given daily to 36 patients, led to hematologic, neurologic or symptomatic relapse in 14, after periods varying from 15 to 49 months of therapy.

The authors conclude that combined oral administration of vitamin B₁₂ and folic acid is inadequate in pernicious anemia but that parenteral vitamin B₁₂ is an adequate substitute for liver. Neurologic relapse can be prevented with more frequent doses of vitamin B₁₂.

*Hekloen Institute for Medical Research of
Cook County Hospital
Chicago III*

21 Prognosis in treated anaemia, Brit M J 1 297 298 Feb 2 1957 (in Any Questions?)

Q—What is the prognosis for patients with pernicious anaemia treated with adequate amount of vitamin B₁₂? Are they ever cured in the sense that treatment is no longer necessary?

A—The introduction of vitamin B₁₂ (cyanocobalamin) is too recent for reliable statistics on survival and morbidity among patients with pernicious anaemia treated with the pure vitamin. Clinical observation however suggests that such patients have an expectation of life and a morbidity no worse than that of the general population. In fact it has been suggested that they may be better off because they come under review every month or two when they receive their injections.

In his presidential address to the Manchester Medical Society last October Dr J F Wilkinson reviewed a personal

series of 2211 cases of pernicious anaemia seen over the past 28 years. Of course, only in the last few years would pure vitamin B₁₂ have been available for the treatment of these patients but the mortality in this series was only 0.83 per cent per year. Almost all died from conditions other than pernicious anaemia but, of those who died of cancer, in about half the primary [neoplasm] was in the stomach. In Dr Wilkinson's experience the expectation of life for the patients in this regularly supervised group of patients was rather better than for people without pernicious anaemia. Of the 1550 patients still attending his clinic no fewer than 758 were over 70 years of age, 283 over 80 years, 39 over 90 and one over 100.

Patients with pernicious anaemia are never cured in the strict sense of the word, though if adequately treated their lives are in every respect as if they had been. The chief pathological change is the atrophy of the gastro-intestinal mucosa which so far has proved irreversible. Very occasionally a patient who discontinues treatment remains well for months or even years before relapsing but these are exceptional cases and afford no general grounds for the discontinuance of treatment. Treatment must be for life.

22 Dosage of vitamin B₁₂, J A M A 163 998, March 16, 1957 (in Queries and Minor Notes)

A physician inquires whether vitamin B₁₂ in doses of 1000 mcg is more effective than in doses of 100 mcg.

Answer—Intramuscularly given doses of 1000 mcg (1 mg) of cyanocobalamin are far in excess of those required in the proper treatment of pernicious anaemia. The paper written for The Council by Unglaub W G *et al* (J A M A 161 623 June 16, 1956) is cited which states:

The amount of vitamin B₁₂ required for maintenance therapy of patients with pernicious anaemia is approximately 1 to 2 mcg daily. The giving of 100 mcg intramuscularly at monthly intervals has been

found satisfactory for most patients. The use of larger doses given at longer intervals does not seem advisable since the amount of vitamin excreted in the urine increases as the dosage is raised. High oral doses of vitamin B₁₂ (in milligrams rather than micrograms) are not recommended for routine use in pernicious anemia.

- 23 Lowther C. P. Alexander W. D. and Hendry E. B. Oral treatment of pernicious anaemia. *Lancet* 1: 495-497, March 6, 1954.

Twenty patients with pernicious anemia in remission and controlled adequately with injections of vitamin B₁₂ or purified liver extract have since been treated satisfactorily with an oral preparation which contains 1.5 Gm. of hog pyloric mucosa and 7.5 mcg. of vitamin B₁₂ per tablet. Two tablets daily were given to 10 patients; the other 10 received four tablets. Subacute degeneration of the cord was present in 3 patients. 1 had active pulmonary tuberculosis and received streptomycin and PAS (para aminosalicylic acid) throughout the six months of the study. There was no evidence of hematologic or neurologic relapse after six months' continuous treatment.

*Western Infirmary
Glasgow, Scotland*

- 24 Meulengracht E. Treatment of pernicious anaemia with very small quantities of pyloric mucosa and vitamin B₁₂. *Brit. M. J.* 1: 838-841, April 10, 1954.

A commercial preparation for oral use containing dried pyloric mucosa from pig stomach and vitamin B₁₂ proved effective in pernicious anemia. Initial dosage was 300 mg. of dried mucosa and 30 mcg. vitamin B₁₂. Maintenance dose has not yet been determined. The mucosa alone 100 or 200 mg. three times daily had a definite but slight anti-anemic effect in patients with untreated pernicious anemia. When these or smaller dosages were

given with 10 to 15 mcg. of oral vitamin B₁₂, there was a strong anti-anemic effect. *Bi. Pøhlerg Hospital and Biological Chemistry Laboratories of the Medicinalco, Copenhagen, Denmark.*

- 25 Brücher H. Oral treatment of pernicious anemia with a compound containing vitamin B₁₂ and pyloric mucosa. *Deutsche med. Wchnschr.* 79: 1726-1728, Nov. 12, 1954 (abstr. *J. A. M. A.* 157: 545-546, Feb. 5, 1955).

Vitamin B₁₂ 5 mcg. and powdered pyloric mucosa 130 mg. combined in an oral tablet were given to 27 pernicious anemia patients twice daily. Bone marrow became normal in all patients. *Stuttgart, Germany.*

- 26 Bethell F. H. Castle W. B. Schwartz S. O. and Wintrobe M. M. I. The use of intrinsic factor B₁₂ combinations in the treatment of pernicious anemia. *Blood* 10: 377-379, April 1955 (in *Panels in Therapy*).

In a panel discussion on pernicious anemia treatment, Dr. F. H. Bethell stated that the more nearly physiologic method of providing vitamin B₁₂ is by its daily oral administration together with intrinsic factor. For this purpose only U. S. P. official preparations to which U. S. P. unitage has been assigned should be used. Such therapy is suitable for intelligent, dependable patients who do not have chronic intestinal disorders or severe neurologic damage. Injections at stated intervals insure the patient's receiving the therapy and returning for periodic observation.

Dr. W. H. Castle preferred use of parenteral injection of vitamin B₁₂ or Liver Injection U. S. P. as it is more certain, more convenient, and less expensive than oral therapy. Adequate parenteral maintenance therapy of pernicious anemia requires only that the patient receive 1 cc. of Vitamin B₁₂ Injection U. S. P. containing 30 mcg. of vitamin B₁₂ in four week intervals.

Objection to the routine use of intrinsic factor and vitamin B₁₂ was made by Dr S O Schwartz on the basis that no long term studies substantiating hematologic control or prevention of neurologic complications are available. He recommends using intrinsic factor and vitamin B₁₂ only in cases of sensitization to parenteral liver and vitamin B₁₂.

Since patients can be maintained in excellent remission by injections, Dr M M Wintrobe stated that it has not been necessary so far to use the intrinsic factor vitamin B₁₂ combinations in the treatment of pernicious anemia. He pointed out that danger of relapse due to the patient's omitting medication is great.

Dr W Dameshek, commenting as the moderator, agreed with Dr Bethell that continued oral medication seems more distinctly physiologic since a small though definite amount of the deficient material is supplied daily. Giving an injection even every month or two might be viewed as a distinctly unphysiologic means of supplying vitamin B₁₂. In the patient with pernicious anemia in remission who can afford the procedure, it has been his custom to give an injection of liver extract or vitamin B₁₂ once every month or two or three and have him take a pill of one of the intrinsic factor vitamin B₁₂ preparations every day.

*University of Michigan Medical School
Ann Arbor, Mich.*

*Thorndike Memorial Laboratory, City Hospital
Boston, Mass.*

*Cook County Hospital
Chicago, Ill.*

*University of Utah Medical School
Salt Lake City, Utah.*

- 27 Blackburn E K, Cohen, H and Wilson O M. Oral treatment of pernicious anaemia with a combined vitamin B₁₂ and intrinsic factor preparation. *Brit M J* 2 461-463, Aug 20 1955.

Five patients with newly diagnosed Addisonian pernicious anemia were treated initially with 30 mcg of crystalline vita-

min B₁₂ and 150 mg of intrinsic factor per day given orally. Four patients showed satisfactory response and one who did not respond later improved on parenteral vitamin B₁₂. A group of 12 patients who had had maintenance therapy with parenteral vitamin B₁₂ for at least a year were then given 6 mcg of vitamin B₁₂ and 30 mg of intrinsic factor daily for one year. They were compared with 12 other patients of similar age and clinical state who were kept on parenteral vitamin B₁₂ therapy. During the second six months of the trial year the patients on the oral preparation showed declines in hemoglobin and red cell counts but the symptomatic and clinical state remained the same. One patient not in the series above showed signs of subacute degeneration of the cord on maintenance therapy with the oral preparation but improved after injections of vitamin B₁₂.

The authors believe that the variable activity of the intrinsic factor may account for the erratic response to the oral preparation. They point out that oral maintenance therapy is at present much more expensive than parenteral. Patients however generally preferred oral therapy.

*University of Sheffield and
Sheffield Royal Infirmary
Sheffield, England.*

- 28 Conley C L and Krevans, J R. New developments in the diagnosis and treatment of pernicious anemia. *Ann Int Med* 43 758-766 Oct 1955.

In pernicious anemia satisfactory remissions have been obtained using preparations containing vitamin B₁₂ and intrinsic factor. However, further extensive clinical trials are required to establish that these preparations are as reliable as is the parenteral injection of vitamin B₁₂. Satisfactory responses can be obtained with very large amounts of vitamin B₁₂ administered orally without intrinsic factor. The authors have treated 45 patients with oral vitamin B₁₂ alone. The initial dose ranged from 3,000 mcg to 10,000 mcg. In most cases the hematologic and

clinical response to these large oral doses equalled that obtained with 30 mcg of parenteral vitamin B₁₂. Remissions were sustained with oral doses of 1 000 mcg weekly.

Measurements of vitamin B₁₂ level in serum in 7 pernicious anemia patients maintained on oral vitamin B₁₂ compared to those in patients receiving vitamin B₁₂ intramuscularly and to those in normal subjects indicated that doses of 1 000 mcg orally are probably suboptimal and higher doses would be necessary for tissue saturation.

It is emphasized that only a small percentage of orally administered vitamin B₁₂ can be absorbed by pernicious anemia patients and their ability to absorb the vitamin varies. The complete effectiveness of monthly parenteral therapy using much smaller doses indicates that it is the preferred method of administration for most patients with pernicious anemia particularly those with neurologic symptoms. However if oral therapy is used crystal line vitamin B₁₂ alone in milligram doses appears as effective as smaller amounts of the vitamin combined with intrinsic factor.

*Johns Hopkins University and Hospital
Baltimore Md*

- 29 Chalmers J N M and Hall Z M
Treatment of pernicious anaemia with oral vitamin B₁₂ without known source of intrinsic factor *Brit M J* 1 1179 1181, May 22 1954

Oral vitamin B₁₂ in amounts ranging from 9 000 mcg in a single dose to repeated daily doses of 50 mcg was administered without known source of intrinsic factor to 12 patients with pernicious anemia in relapse and to 3 gastrectomized patients with similar symptoms. Oral vitamin B₁₂ alone proved to be hemopoietic when given either in large single doses of 2 000 mcg or more or in repeated daily doses of 50 mcg. The uniformly good results obtained with daily oral doses of 50 mcg may be related to the fact that all patients took the dose when fasting and

had no food for six hours afterward. The investigators conclude: "Our results demonstrate again that extrinsic sources of intrinsic factor are not essential for utilization of vitamin B₁₂ and that oral therapy with the vitamin alone may be most effective when given to fasting patients."

*St George's Hospital and Medical School
London England*

- 30 Reisner E H Jr Weiner L Schittone M T and Henck E A
Oral treatment of pernicious anemia with vitamin B₁₂ without intrinsic factor *New England J Med* 253 502 506 Sept 22, 1955

For periods from 12 to 28 months 43 patients with pernicious anemia were maintained on oral vitamin B₁₂ without intrinsic factor. Doses of 1 mg vitamin B₁₂ were given at intervals ranging from twice weekly to once every four weeks; the majority receiving treatment at one or two week intervals.

The authors state: "These studies indicated to our satisfaction that regular oral doses of 1 mg of vitamin B₁₂ would maintain patients with pernicious anemia in satisfactory hematologic and neurologic remission when given at weekly intervals."

*New York University Post Graduate Medical School
New York NY*

- 31 Estren S and Wasserman L R
Pernicious anemia. I. Remission by small oral doses of purified vitamin B₁₂ *Proc Soc Exper Biol & Med* 91 499 503 March 1956

Vitamin B₁₂ 50 to 168 mcg was given orally one to three times daily without intrinsic factor to 9 patients with pernicious anemia in relapse and to 1 with megaloblastic anemia. Maximal reticulocytosis (within 8 to 9 days) and hematologic response with reversion of bone marrow to normoblastic erythropoiesis occurred in 3 cases of pernicious anemia and 1 of megaloblastic anemia. In

5 pernicious anemia cases response was submaximal in 1 none occurred Remission extended four to six months after therapy ended Two patients who responded initially failed to respond to this regimen when again in relapse

The authors state that the results support the suggestion that in pernicious anemia is quantitative rather than a qualitative change in gastric secretion occurs, and that varying amounts of intrinsic factor in pernicious anemia patients may cause varying responses to oral vitamin B₁₂ A trial of vitamin B₁₂ alone should precede trial of a vitamin B₁₂ plus intrinsic factor preparation

*Mount Sinai Hospital
New York N Y*

- 32 Monto R W and Rebusk, J W
Observations on the mechanism of intranasal absorption of vitamin B₁₂ in pernicious anemia, *Blood* 10 1151-1155, Nov 1955

Vitamin B₁₂ (without intrinsic factor) was administered intranasally to 32 patients with pernicious anemia in relapse and to 55 patients in remission The vitamin produced equal response when given in saline solution, in lactose powder, or when used as crystals Doses of 100 mcg brought about maximal reticulocyte response in the patients in relapse and provided adequate maintenance levels in patients in remission The hematologic gastrointestinal and neurologic improvement was comparable to that noted after parenteral therapy

Nasal washings taken from a normal subject after instillation of 150 mcg of vitamin B₁₂ were administered to one patient by means of a gastric tube There was no significant increase in the reticulocyte count

Urinary bioassay for vitamin B₁₂ performed in a patient who received 200 mcg of vitamin B₁₂ intranasally showed that 12 per cent of the administered dose was excreted These studies suggest the direct absorption of the vitamin B₁₂

through the respiratory mucosa rather than binding or combining with a mucoprotein (intrinsic factor) of the nasal secretions

*Henry Ford Hospital
Detroit Mich*

- 33 Monto R W, Rebusk, J W and Howell, J T The single intra nasal application of vitamin B₁₂ crystals in pernicious anemia A clinical and laboratory appraisal of two patients *J Lab & Clin Med* 44 900, Dec. 1954 (in Soc Proc)

In a patient with an initial erythrocyte count of 2.2 millions 150 mcg of vitamin B₁₂ crystals applied to the nasal mucosa caused reticulocytosis of 44.5 per cent on the 6th day Complete remission (hemoglobin 16.5 Gm red blood count 5.65 millions) was obtained for three months without further treatment

A patient in relapse with an erythrocyte count of 2.01 millions was given 200 mcg vitamin B₁₂ crystals on the nasal mucous membrane A reticulocytosis of 43.4 per cent occurred on the 7th day A complete clinical remission was obtained with an increase of erythrocytes to 4.12 millions in a two-month period without further treatment.

It was concluded that a single application of vitamin B₁₂ to the nasal mucosa effected complete clinical and laboratory response in these 2 pernicious anemia patients The nonirritating vitamin B₁₂ crystals were rapidly absorbed by the nasal mucous membranes Absorption of the vitamin B₁₂ by the nasal mucosa is probably not dependent upon binding with intrinsic factor

Detroit Mich

- 34 Monto, R. W and Rebusk, J W Nasal instillation and inhalation of crystalline vitamin B₁₂ in pernicious anemia, *A M A Arch Int Med.* 93 219 230, Feb 1954

Since the success of parenteral injection of vitamin B₁₂ is attributed to ready

access to blood and lymph capillaries and since one of the largest capillary beds in contact with the external environment is that of the pulmonary circulation in halation administration of vitamin B₁₂ seemed logical. The similarity of the mucosa of the nose and the tracheobronchial tree suggested nasal instillation of this vitamin. Concentrations of 15 to 200 mcg of vitamin B₁₂ per cc of isotonic sodium chloride solution were used. A concentration of 100 mcg per cc gave maximum hemopoietic effect. For administration as dust, 1 000 mcg of vitamin B₁₂ in 0.1 cc. by volume of lactose powder was used.

Both inhalation and nasal instillation were used in treating 12 patients with pernicious anemia in relapse and 20 with pernicious anemia in remission. No evidence of toxicity or sensitivity at the local site was observed. A satisfactory hematologic and clinical response comparable to that expected from parenteral administration was obtained in the patients in relapse and the condition of patients in remission had been maintained for as long as 18 months at the time of writing.

*Henry Ford Hospital
Detroit, Mich.*

35. **Isaacs M. C. G. and Shubert S.**
The treatment of pernicious anaemia by insufflation of vitamin B₁₂. *Lancet* 1 341 343 Feb 13, 1954

Five patients with pernicious anemia were successfully treated with vitamin B₁₂ in the form of snuff for insufflation. Doses were of the same order as those commonly used for intramuscular administration.

*University of Manchester and
Royal Infirmary
Manchester, England*

PERNICIOUS ANEMIA ANALYTICAL STUDIES

36. **Hamilton H. E., DeGowin E. L., Sheets R. F., Janney C. D. and Ellis J. A.** Studies with inagglutinable erythrocyte counts. VI. Acceler-

ated destruction of normal adult erythrocytes in pernicious anemia: contribution of hemolysis to the oligocythemia. *J. Clin. Investigation* 33 191 205 Feb 1954

Transfusions of fresh normal erythrocytes to patients with pernicious anemia suggest that vitamin B₁₂ deficiency adversely affects normal circulating adult erythrocytes. Pretreatment with 15 mcg of vitamin B₁₂ daily for 9 days appeared sufficient to prevent hemolysis of transfused erythrocytes, but treatment for 3 days was apparently insufficient. Daily administration of 15 mcg of vitamin B₁₂ for 23 days protected transfused erythrocytes from random destruction for only 20 days after cessation of therapy.

In 4 of 6 patients with untreated pernicious anemia the oligocythemia could be entirely accounted for by random erythrocyte destruction as measured by the loss of normal transfused cells. In 2 other patients maturation arrest in the bone marrow was a factor in the oligocythemia, but part of it could be attributed to abnormally rapid destruction of erythrocytes.

*State University of Iowa
Iowa City, Iowa*

37. **Glazer H. S., Mueller J. F., Jarrold T., Sakurai K., Will J. J. and Vilter R. W.** The effect of vitamin B₁₂ and folic acid on nucleic acid composition of the bone marrow of patients with megaloblastic anemia. *J. Lab. & Clin. Med.* 43 905 913 June 1954

Ribonucleic and desoxyribonucleic acid (RNA and DNA) thymine (T) and uracil (U) concentrations in the bone marrow of 11 patients with pernicious anemia and in 1 with sprue were determined by paper chromatography before and after treatment with one or more of the following: ascorbic acid, vitamin B₁₂, folic acid, and folinic acid. Similar determinations were made on 19 normal subjects.

The RNA/DNA and U/T ratios were higher than normal in patients with mea-

aloblastic anemia, the relative amount of thymine was lower than normal. After adequate treatment with hemopoietic vitamins the RNA/DNA and U/T ratios fell and the relative amount of thymine rose to normal as the bone marrow became normal cytologically.

The results suggest that in hemopoietic tissues, vitamin B₁₂, folic acid and ascorbic acid promote formation of thymine and its desoxyribotides and depletion of uracil and its nucleotides. There is also evidence that a greater percentage change takes place in the pyrimidine concentrations than in the nucleic acid concentrations. This may indicate that a qualitative effect on the composition of the nucleic acids is initiated by these vitamins.

The following daily dosages were employed: (1) ascorbic acid orally 1,000 mg; (2) vitamin B₁₂ intramuscularly, 0.25 mcg or 5 to 30 mcg; (3) folic acid intramuscularly 0.5 or 10 mg; (4) folinic acid intramuscularly, 1 mg.

University of Cincinnati
College of Medicine
Cincinnati, Ohio

- 38 Sandberg A A, Eik Nes K, Nelson D H, Palmer J G, Cartwright, G E and Wintrobe, M M. Adrenocortical function and metabolism of 17 hydroxycorticosteroids in pernicious anemia. *New England J Med* 251:169-174 July 29, 1954.

Adrenocortical function and metabolism of 17 hydroxycorticosteroids in pernicious anemia were studied by measurement of the blood 17 hydroxycorticoids in 8 patients with pernicious anemia in relapse and after vitamin B₁₂ therapy in normal controls and in 1 patient with achylia gastrica.

There was no evidence that adrenocortical function or steroid metabolism is impaired in patients with pernicious anemia in relapse. Normal 17 hydroxycorticoid levels were found during relapse (except in 2 severely ill patients whose 17 hydroxycorticoid levels were elevated) and

after vitamin B₁₂ therapy. The adrenal cortex responded normally to corticotropin administration. One patient with pernicious anemia in relapse was given hydrocortisone intravenously and 2 were given cortisone intravenously. The rate of disappearance of 17 hydroxycorticosteroids from the plasma was normal.

After oral administration of 200 mg of hydrocortisone acetate suspension to 4 patients with pernicious anemia and 200 mg of hydrocortisone suspension to 1 patient with pernicious anemia, plasma 17 hydroxycorticoid levels were elevated for prolonged periods. The magnitude of the elevation was not significantly influenced by vitamin B₁₂.

A patient with achylia gastrica and no evidence of anemia showed the same prolonged 17 hydroxycorticosteroid level after oral hydrocortisone as the patients with pernicious anemia. Ten patients with pernicious anemia were given 200 mg of hydrocortisone suspended in 150 cc of normal gastric juice. The 17 hydroxycorticosteroids were lower than when hydrocortisone was given without gastric juice.

The data derived from the study of the patient with achylia gastrica without pernicious anemia indicate that the presence of achylia per se rather than its metabolic effects, is the important factor in the response. Failure of vitamin B₁₂ therapy to modify the results further implicates the achylia.

Salt Lake City, Utah

- 39 Pollycove M, Apt, L and Ross J F. Erythropoiesis and the movement of iron in patients with pernicious anemia. *Clin Research Proc* 3:92-93, April 1955.

The movement of iron in 7 patients with pernicious anemia before and after initial therapy with vitamin B₁₂ was studied with the use of tracer doses of Fe⁵⁹. Before treatment the rate of removal of iron from the plasma was 4 to 6 times normal, only 10 to 20 per cent was incorporated into circulating erythrocytes (nor

mal 90 to 100 per cent) and the iron initially accumulated with abnormal rapidity in the bone marrow and spleen. After retention in the marrow for several days most of the iron gradually moved to the liver.

Three days after the initiation of vitamin B₁₂ therapy radioactive iron was rapidly depleted from the liver and bone marrow. A simultaneous sharp increase of radioactive iron within circulating erythrocytes occurred.

These studies demonstrate a slight destruction of circulating erythrocytes but emphasize the predominance of arrested erythrocyte hemoglobinization in producing pernicious anemia. They also suggest that the abnormal increment of fecal urobilinogen in this disease is probably derived from the breakdown of heme or other porphyrins within the bone marrow.

*Boston V A Hospital and
Tufts College Medical School
Boston, Mass.*

- 40 Rabiner S F and Lichtnam H C. Studies on the relationship between the peripheral blood counts and the total body red cell volume. *AMA Arch Int Med* 99:474-480 March 1957.

It has been shown previously that in normal persons there is a relatively constant relationship between the hematocrit of peripheral venous blood and the circulating red blood cell volume per kilogram of body weight (CRBCV/kg).

Thirteen patients with Addisonian pernicious anemia were studied by determining the circulating red blood cell volume using isotopically tagged red blood cells.

A poor correlation between the peripheral venous hematocrit and the circulating red blood cell volume per kilogram body weight was found in patients with pernicious anemia in relapse and during regeneration. In some instances the venous hematocrit was falsely high and other times falsely low. In a few selected cases this poor relationship was also found

in patients with Addisonian pernicious anemia during remission.

The data indicate that the degree of blood regeneration in response to specific therapy cannot always be estimated by the changes in peripheral blood cell counts. The hematologic response as determined by changes in CRBCV/kg of body weight may be greater than that indicated by the changes noted in the venous blood. In some persons the clinical state seems to be as useful a guide in evaluating the response to therapy as is the estimation of the red blood cell volume.

*St. Louis University of
New York College of Medicine
New York, N. Y.*

- 41 Thomas E D and Lochte H L Jr. In vitro observations on oxygen consumption, heme synthesis and desoxyribonucleic acid synthesis by pernicious anemia bone marrow. *Clin Res Search Proc* 5:145-146 April 1957.

The nature of the biochemical defect in pernicious anemia (P.A.) is still not understood. We have studied P.A. bone marrow by the following techniques: (1) Oxygen consumption, (2) Heme synthesis as measured by the rate of incorporation of C¹⁴ glycine into heme, and (3) Desoxyribonucleic acid (DNA) synthesis by measurement of the rate of incorporation of C¹⁴ formate into thymine.

Utilizing these methods it was found that P.A. bone marrow synthesizes INA at a greater rate in normal serum than in P.A. serum. Addition of vitamin B₁₂ to P.A. bone marrow in P.A. serum accelerates the rate of DNA synthesis. B₁₂ and folic acid have no effect on oxygen consumption or heme synthesis. Folic acid and citrovorum factor accelerate markedly DNA synthesis in some marrow samples and show no effect in others. P.A. serum has no demonstrable inhibitory effect on normal bone marrow. From these studies it is concluded that vitamin B₁₂ and folic acid are specifically con-

cerned with DNA synthesis that B₁₂ has a direct action on the marrow cells and that folic acid affects some P A marrows but not others

These studies provide evidence of the importance of B₁₂ in DNA synthesis by human cells. The techniques described make it possible to apply the data from numerous microbiologic studies to human cells

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NUTRITIONAL MACROCYTIC ANEMIAS

- 42 Das Gupta C R., Chatterjea, J B and Basu P Vitamin B₁₂ in nutritional macrocytic anaemia, Brit M J 2 645 649, Sept 19 1953

In a Calcutta hospital 22 Indian patients with uncomplicated nutritional macrocytic anemia were treated with vitamin B₁₂. Fifteen received intramuscular therapy 1 received oral therapy and 6 received both at different times. In general maximum response followed 200 to 300 mcg of intramuscular vitamin B₁₂ but dose and response had no distinct correlation. There was no correlation between response to oral vitamin B₁₂ with either dose or state of gastric acidity. Initial improvement occurred in 14 cases but was sustained in only 5. Reticulocytosis was inadequate and was not correlated with the degree of improvement. Folic acid and crude liver extract given to patients who relapsed gave adequate or better results in most cases.

The authors state that these studies definitely indicate that nutritional macrocytic anemia involves deficiency of both folic acid and vitamin B₁₂. Deficiency of folic acid however is described as the major and primary factor and that of vitamin B₁₂ the minor and secondary factor in most cases.

School of Tropical Medicine
Calcutta India

- 43 Stahle, T D Megaloblastic anemia and vitamin B₁₂, Maandschr v kin dergeneesk 21 396, Dec 1953 (abstr J A M A 154 1137, March 27, 1954)

A child with megaloblastic anemia recovered after treatment with intramuscular vitamin B₁₂ followed by oral vitamin B₁₂. The daily dosage by both routes was 5 mcg. Maintenance therapy with vitamin B₁₂ was not necessary. It is believed that nutritional deficiencies during the war were responsible for the anemia.

- 44 Watson, R J, Lichtman, H C Mesite J, Ellison, R R, Conrad, H and Ginsberg V Clinical studies with the citrovorum factor in megaloblastic anemia. Am J Med 17 17 28 July 1954

Five patients with nutritional megaloblastic anemia had hematologic remissions after therapy with citrovorum factor (CF). Four had previously failed to improve when given vitamin B₁₂. Two patients apparently had dual deficiencies of both vitamin B₁₂ and CF. One had addisonian pernicious anemia and a superimposed deficiency of CF. The other had cirrhosis of the liver and a nutritional megaloblastic anemia. The latter showed a good hematologic remission initially when given vitamin B₁₂ but subsequently relapsed and responded only to the administration of CF.

Treatment of a patient with addisonian pernicious anemia with CF resulted in acute combined system disease which cleared up promptly after parenteral administration of vitamin B₁₂. The use of CF or PGA (pteroylglutamic acid) is undoubtedly contraindicated in addisonian pernicious anemia. It is possible that the hematologic response to these agents may further deplete the stores of vitamin B₁₂, accelerating the development of combined system disease. In megaloblastic anemias with associated gastric achlorhydria vitamin B₁₂ therefore is the drug of choice. When hydrochloric acid is found

in the gastric juice treatment with CF or PGA is indicated

State University of New York
New York N Y
Kings County Hospital
Brooklyn N Y

- 45 Gosselin B and Morissette L. Le traitement des anémies macrocytaires par la vitamine B₁₂ orale [Treatment of macrocytic anemias by oral vitamin B₁₂] Union méd. du Canada 83 1238-1241, Nov 1954

A patient with nutritional macrocytic megaloblastic anemia was successfully treated with a preparation which contains per capsule 100 mcg of vitamin B₁₂, 75 mg of extract of the duodenal mucosa and 175 mg of folic acid. At the time the case was reported the patient had resumed his work and was being maintained in perfect health by a vitamin B₁₂ preparation.

Despite the efficacy of this oral preparation, the authors are not convinced that oral treatment is superior in general to intramuscular administration since an injection every two or three weeks maintains the patient in good condition. When injections are not feasible however oral treatment will render great service.

Hôpital Notre-Dame
Montreal Canada

- 46 Polycove, M Apt L and Colbert M. J. Pernicious anemia due to dietary deficiency of vitamin B₁₂. Clin Research Proc. 3 28 Feb 1955

A man of 60 who had scrupulously avoided all foods of animal origin for at least 8 years was admitted with a severe macrocytic anemia associated with a megaloblastic bone marrow. The serum vitamin B₁₂ level was 70 micromicrograms per cc and hematologic response to parenterally administered vitamin B₁₂ was characteristic. The patient's gastric juice contained free hydrochloride and when it was administered with Co⁵⁷ vitamin B₁₂ to a patient with pernicious ane-

mia normal absorption resulted as indicated by low fecal elimination and a high hepatic uptake. The patient himself exhibited normal absorption of Co⁵⁷ vitamin B₁₂ after remission of the anemia. Thus this patient in spite of adequate intrinsic factor apparently developed vitamin B₁₂ deficiency as the result of a prolonged dietary deficiency of it.

Tufts College Medical School and
Boston V A Hospital
Boston Mass

- 47 Wokes F, Badenoch J and Sinclaire H M. Human dietary deficiency of vitamin B₁₂. Am J Clin Nutrition 3 375-382 Sept Oct 1955

The authors compare the dietary intake of calories, protein and vitamin B₁₂ and describe vitamin B₁₂ deficiency symptoms in American, Dutch and British vegans (vegetarians who do not eat dairy products). The commonest and earliest symptoms of vitamin B₁₂ deficiency were oral paresthesia, developed more slowly. Amenorrhea and menstrual disorders occurred in 8 of 22 British women. Nervous symptoms were common as were pains and stiffening of the spine and back. The incidence of deficiency symptoms was lower in the Dutch than in the British and did not appear among the 26 Americans all of whom had been vegans five years or longer. Serum vitamin B₁₂ levels showed a steady fall during the first 7 years, a return to near normal between 7 and 10 years, and then a more gradual descent. In vegans with anemia correlation of the red cell counts with vitamin B₁₂ levels gave an average curve unlike that seen in pernicious anemia but similar to that seen in subacute combined degeneration of the cord. This may reflect the large ingestion of folic acid.

The nervous system symptoms observed in some vegans might have been due to chronic toxicity caused by an increase in the blood concentration of a keto acid derivative of tyrosine. Urinary excretion and blood levels of thiocyanates

were also increased and were further stimulated after tyrosine ingestion

The results of the investigation indicate the occurrence in man of a dietary deficiency of vitamin B₁₂ similar to that encountered in animals living on a diet free from animal protein. The poor rate of growth and development in such animals can be improved by addition of vitamin B₁₂ provided that their diet is not deficient in total protein

The authors attribute the varying incidence of deficiency symptoms in the Dutch, British and Americans to the variations in total protein intake in the three groups. The British who had the lowest intake (7 per cent) had the highest incidence of deficiency while the Americans who ingested the largest amount of protein (10.4 per cent) had no clinical deficiency symptoms

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King's Langley, England
Radcliffe Infirmary and
University of Oxford
Oxford, England*

ANEMIAS OF PREGNANCY

- 48 Das Gupta C R, Chatterjee J B and Basu P. Vitamin B₁₂ in macrocytic anemia in pregnancy. *Indian Med Gaz* 88:102-105, 1953 (abstr. *Blood* 10:667-668, June 1955)

Twenty one cases of macrocytic anemia in pregnancy: 13 with megaloblastic and 8 with normoblastic bone marrow were treated with vitamin B₁₂ by the oral or the intramuscular route. In both groups the response was better by the intramuscular route. Initial response was good in 70 per cent of cases with megaloblastic marrow and in 80 per cent of cases with normoblastic marrow. The good initial response was not however sustained and continuation of vitamin B₁₂ therapy did not usually produce further improvement of blood picture but administration of folic acid or crude liver extract did. The authors suggest that an

average case of macrocytic anemia in pregnancy represents combined deficiency of folic acid and vitamin B₁₂

*School of Tropical Medicine
Calcutta, India*

- 49 Clarke, J P and Essig, L L. Megaloblastic anemia of pregnancy. *Am J Obst & Gynec* 67:367-372, Feb 1954

Case histories of 3 patients with megaloblastic anemia of pregnancy are presented. The first patient received intramuscular vitamin B₁₂ (30 mcg daily) for two days prior to delivery. Vitamin B₁₂ rather than folic acid was given in this case because of lack of appreciation of the greater specificity of folic acid in this condition. The authors describe the response to vitamin B₁₂ as 'inadequate'. The other 2 patients were treated with folic acid 10 mg and 15 mg by mouth daily respectively. Both showed an adequate reticulocyte and clinical response.

*University of Colorado
School of Medicine
Denver, Colo*

- 50 Moore H C, Lillie, E H and Gatenby, P B. The response of megaloblastic anaemia of pregnancy to vitamin B₁₂. *Irish J Med Sc* 6th Series No 351:106, March 1955 (abstr. *Am J Obst & Gynec* 71:1163, May 1956)

Seventeen cases of megaloblastic anemia of pregnancy at the Rotunda Hospital were treated with vitamin B₁₂. Six patients also received iron therapy. Fecal fat analysis was performed during a four-day period with the patients on a standard diet containing 70 grams of fat and 70 grams of protein daily. Thirteen patients had a reticulocyte response to therapy. The four cases which did not respond to vitamin B₁₂ therapy responded favorably to subsequent folic acid therapy. The authors conclude that there are two groups of megaloblastic anemia of pregnancy depending on the response to vitamin B₁₂ and not all cases can be regarded as folic acid deficient anemias.

51. Badenoch J Callender ■ T Evans
J R Turnbull A L. and Wits L J
Megaloblastic anemia of pregnancy
and the puerperium Brit M J 1
1245-1247 May 21 1955

In 9 patients with megaloblastic anemia of pregnancy and the puerperium intrinsic factor secretion fat absorption and serum vitamin B₁₂ levels were normal. Of the 4 examined before delivery 2 responded to folic acid and 2 did not respond either to folic acid or to vitamin B₁₂ therapy. The 3 patients studied during the puerperium seemed to respond to folic acid or to vitamin B₁₂ but these recoveries might have been spontaneous remissions which are common after delivery. Of 2 patients with severe anemia six weeks after delivery 1 responded to folic acid and 1 to vitamin B₁₂.

The authors attribute the variable findings to toxemia and infection, hemorrhage multiple or rapidly repeated pregnancies or to poor diet and gastrointestinal upsets. However when pregnancy alone seems to induce anemia the cause appears to be the development of resistance to vitamin B₁₂ and folic acid.

*Radcliffe Infirmary
Oxford England*

52. Berry C G Anaemia of pregnancy
in Africans of Lagos Brit M J 2
819-823 Oct 1 1955

A study of the anemia of pregnancy among African women in Lagos included 30 patients. The diet of these people consists mainly of starchy vegetables palm and groundnut oil with little meat eggs milk or fruit. The red cell counts in the 30 patients ranged from 0.69 million to 3.4 million per cu mm. The hemoglobin concentration ranged from 2.5 to 11 Gm per 100 cc. Leukocyte counts ranged from 4,300 to 19,600 per cu mm. Stool examinations showed worm infestations in many of the patients which undoubtedly aggravated the anemia.

Vitamin B₁₂ 100 or 200 mcg initially followed by 20 or 50 mcg at weekly inter-

vals was administered in 13 patients. Of the 9 patients treated during pregnancy 4 responded rapidly and 3 more slowly. Clinical improvement in all these was striking. Of the 4 patients treated after delivery 2 showed no response to vitamin B₁₂ or any other agent and 2 patients responded well. However this response could not be attributed completely to vitamin B₁₂ because of the possibility of spontaneous remission following delivery.

The author states that although the primary deficiency among these women may have been folic acid response to folic acid may not be possible until the secondary deficiency of vitamin B₁₂ is corrected. After parturition the slow spontaneous recovery which occurs as folic acid again becomes available may be accelerated by administration of vitamin B₁₂.

*St James' Hospital
London England*

53. Lowenstein L. Pick C and Philpott, R. Megaloblastic anemia of pregnancy and the puerperium Am J Obst & Gynec 70 1309 1337, Dec 1955

In 18 patients with anemia of pregnancy or the puerperium dietary deficiency was the apparent cause in 12 cases in the other 6 were not definitely known. Treatment consisted of oral or intramuscular vitamin B₁₂ or folic acid or oral folic acid. Many of the patients received more than one of these agents in sequence.

Vitamin B₁₂ was effective by the oral route in 1 patient and by the parenteral route in 3. It failed in 4 patients who were later successfully treated with folic acid. A complete response was obtained with folic acid alone in 1 patient. Folic acid produced good response in 8 patients while 3 had spontaneous remissions.

In June 1953 the authors began routinely prescribing prenatal supplements which included vitamin B₁₂ and folic acid. They believe that this is responsible for

the fact that since then no patients with megaloblastic anemia of pregnancy have been admitted to the hospital

It is concluded that in temperate climates megaloblastic anemia associated with pregnancy or the puerperium may result from a deficiency of folic acid or vitamin B₁₂ or possibly of a third unknown factor

*McGill Medical School and
Royal Victoria Hospital
Montreal Quebec*

- 54 Adams, E B Treatment of megaloblastic anaemia of pregnancy and the puerperium with vitamin B₁₂, Brit M J 2 398-400 Aug 18, 1956

Author's summary The results of a clinical trial using vitamin B₁₂ in 10 patients with megaloblastic anaemia of pregnancy are recorded The appearance of the bone marrow is briefly described

Response was satisfactory in all seven treated after delivery It appeared to be as good with doses of 100 µg as it was when 1 000 µg or more was given, but better results are usually obtained with folic acid

Three patients treated before delivery responded poorly

Because of differences in the appearance of the bone marrow and in response to treatment it seems likely that there are several varieties of megaloblastic anaemia associated with pregnancy

*University of Natal
Durban South Africa*

- 55 Izak G Rachmilewitz M Stein Y, Berkovici, B, Sadovsky, A Aro novitch, J and Grossowicz N Vitamin B₁₂ and iron deficiencies in anemia of pregnancy and puerperium A M A Arch Int Med 99 346-355 March 1957

Of 2 500 women examined during pregnancy or the puerperium 276 were anemic The group was generally malnourished

Repeated hematologic examinations and serum vitamin B₁₂ determinations

were carried out in 100 of the anemic women Of these 30 had hypochromic iron deficiency anemia with low serum iron and normal serum vitamin B₁₂ levels In 11 patients with hyperchromic macrocytic anemia very low vitamin B₁₂ concentrations were found with normal or high serum iron concentrations The remaining 59 had dimorphic anemia, with both low serum vitamin B₁₂ and low iron concentrations

A gradual fall to below 200 micrograms of the serum vitamin B₁₂ concentration took place in most anemic patients during the last trimester of pregnancy, followed by a rise during the two to four weeks following delivery Vitamin B₁₂ deficiency was attributable not only to the utilization of large amounts of the vitamin in the growth of the embryo but also to the fact that the diet during pregnancy as well as during preceding years had been poor in animal proteins

Though it has been generally accepted that megaloblastic anemia of pregnancy responds to crude liver extract and to folic acid but not to vitamin B₁₂ recent reports have cited favorable therapeutic results in some patients treated with vitamin B₁₂

*Hebrew University Hadassah Medical School
Jerusalem Israel*

- 56 Thompson R B Seasonal incidence of megaloblastic anaemia of pregnancy and the puerperium Lancet 1 1171 1172 June 8, 1957 (in Original Articles)

The author suggests that dietary deficiency is a significant factor in megaloblastic anemia of pregnancy in the tropics If this is true more patients may be expected to develop the disorder during winter and spring A study of 100 cases of megaloblastic anemia of pregnancy showed just such an increased incidence in winter and spring

Because much of the supply of folic acid comes from green vegetables it may be that dietary sources in the winter are

inadequate to meet the increased needs of pregnancy Vomiting of pregnancy further decreases intake The reason why patients with the disorder in temperate climates develop a predominantly if not exclusively folic acid deficiency is explained by Vinke and Van der Sar (1956) who point out that healthy people have a store of vitamin B₁₂ which can supply their requirements for many months and sometimes for years The store of folic acid on the other hand can supply the needs of the body for only about a month In a previously well nourished person with increased demands for both substances and with a poor dietary intake folic acid deficiency becomes manifest much earlier than does a deficiency of vitamin B₁₂ In a previously malnourished person with a low store of vitamin B₁₂ however the deficiency is likely to be of both vitamin B₁₂ and folic acid this would obtain in patients developing megaloblastic anaemia in the tropics

The evidence presented here does not warrant the assumption that all cases of megaloblastic anaemia of pregnancy are due to dietary deficiency but it suggests that dietary deficiency is at least an important contributory cause It is interesting that the disorder disappeared from the wards of a maternity hospital in Montreal when an antepartum daily supplement of 4.5 µg of vitamin B₁₂ and 3.0 mg of folic acid was given (Lowenstein *et al* 1955)

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Princess Mary Maternity Hospital
Newcastle upon Tyne England*

ANEMIAS OF INFANCY

- 57 Cartagenova L. So-called pernicious form anemia with megalocytes of children, *Lattante* 24 856 Nov 1953 (abstr A M A. Am J Dis Child 88 689 Nov 1954)

Perniciousiform anemia with megalocytemia was diagnosed in a boy of 13

months and a girl of 12 months who had hemolytic anemia megalocytes in the peripheral blood megaloblastic bone marrow glossitis and gastrointestinal disturbances Favorable results followed treatment with liver extract and vitamin B₁₂ Aside from a few cases described in America practically all cases previously reported have been from Sicily These children were not Sicilian

- 58 Roberto B. Pernicious like anemia and other megaloblastic anemias of infancy *Acta haematol* 11 355 371, 1954 (abstr Blood 9 1122, Nov 1954)

The author discusses the various megaloblastic anemias in infancy He stresses the curable types including pernicious like anemia of Gerbasi It is characterized by symptoms referable to the nervous system and by a response to small amounts of vitamin B₁₂ given orally The disease seems to be due to insufficient vitamin B₁₂ in the food

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Palermo Sicily Italy*

OTHER ANEMIAS

- 59 Kenawy, M R and Hassanein M A. Combined vitamin B₁₂ and folic acid therapy in iron deficiency anaemias *Acta haemat* 10 92 94 1953 (abstr Blood 9 296 March 1954)

Experiments were carried out in cases of hypochromic anemia using a mixture of 30 mcg B₁₂ and 10 mg folic acid for daily injections The average daily increase of hemoglobin in the cases treated with the vitamin B₁₂ and folic acid in addition to the iron therapy was 1.77 per cent and 0.78 per cent in the control group with iron treatment alone

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Cairo Egypt*

- 60 Foy, H and Kondi, A Nutritional and intestinal factors and iron losses in the genesis of tropical anaemias, *Lancet* 1 423-424, April 14, 1956

The etiology and treatment of hypochromic, microcytic iron-deficiency anemias occurring in the tropics are discussed. In the absence of iron deficiency such anemias might be due to intestinal blood loss caused by parasites, defective intestinal iron absorption caused by abnormal calcium phosphorus ratios, or loss of iron through feces, urine or sweat which is estimated to be 1.0 to 1.5 mg per day.

Most patients with hypochromic microcytic iron deficiency anemias responded to the daily oral administration of iron or to iron and protein in some form. Patients who did not respond to this treatment and who had giant stab cells in the urine often responded to 5 mg

of folic acid daily for one week or 80 mcg of vitamin B₁₂ or both.

- 61 Foy H and Kondi, A. Genesis of tropical anaemias, *Lancet* 2 95-96, July 14, 1956 (Letter to Editor)

Microcytic, hypochromic iron-deficiency anemia usually responds to iron but in some cases protein in the form of skimmed milk must also be given. A smaller group of these anemias responds fully only if the patient is given in addition to iron and protein 80 to 100 mcg of intramuscular vitamin B₁₂ in spite of the fact that the vitamin B₁₂ serum level is normal. Perhaps the reason is that in these anemias the vitamin B₁₂ level in the serum must be higher to facilitate protein utilization and metabolism or for the synthesis of methyl and SH groups for hemopoietic purposes.

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NERVOUS SYSTEM

NEUROPATHIES DUE TO VITAMIN B₁₂ DEFICIENCY

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It is concluded that vitamin B₁₂ defi-

ciency may cause neuropathy without hematologic changes. In this case subtotal gastrectomy may have caused the deficiency.

Marzelsborg Hospital
Aarhus Denmark

- 63 Jewesbury E C O. Subacute combined degeneration of the cord and achlorhydric peripheral neuropathies without anaemia. *Lancet* 2 307-312, Aug 14, 1954

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for only two weeks. Thereafter the patient remained well for three years but then developed pernicious anemia.

In the 3 other patients who received adequate parenteral treatment with vitamin B₁₂, pernicious anemia did not develop, neurologic signs did not advance and there was symptomatic recovery. In 2 of these 3 cases giant metamyelocytes were seen in the marrow; this may be one of the earliest signs of pernicious anemia.

Two other patients are described in whom peripheral neuropathy was associated with histamine fast achlorhydria and a normal peripheral blood count. In 1 of these, minimal changes in the sternal marrow also suggested the beginning of pernicious anemia.

Patients with histamine fast achlorhydria are liable to develop vitamin B₁₂ deficiency but the relative susceptibility of the peripheral nerves, spinal cord and marrow is unpredictable. Early treatment and close observation of patients with neurologic manifestations is essential. A few cases of nonanemic peripheral neuropathy with histamine fast achlorhydria may be due to causes other than vitamin B₁₂ deficiency.

The author suggests that the terms pernicious anemia and subacute combined degeneration of the cord be dropped in favor of the more comprehensive term vitamin B₁₂ deficiency. This might be qualified by adding megaloblastic anemia and/or neuropathy (cerebral, spinal or peripheral).

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North Middlesex Hospital
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*Chase Farm Hospital
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*Laton and Dunstable Hospital
Laton, England*

6. Arias I. M., Apt L. and Pollycove M.: Absorption of radioactive vitamin B₁₂ in nonanemic patients with combined-system disease. *New England J. Med.* 253:1005-1010, Dec. 8, 1955.

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Radioactive vitamin B₁₂ was given orally with and without normal human gastric juice and also subcutaneously. Scintillation from the liver was measured 10 days later. The hepatic count after subcutaneous administration was assumed to represent 100 per cent absorption. In the 4 subjects, the hepatic count per minute per microcurie after oral dosage was 0 to 19 per cent of that seen after the subcutaneous dose. When the same oral dose was given with normal human gastric juice, the hepatic count was 59 to 63 per cent of that obtained after injection. Gastric juice from the patients was given orally with radioactive vitamin B₁₂ to other patients with treated pernicious anemia. These patients were also given test doses of vitamin B₁₂ without gastric juice, subcutaneous doses and oral test doses with normal human gastric juice. Hepatic counts were low when the radioactive vitamin or the vitamin plus gastric juice from the patients with combined system disease was given. Counts after oral ingestion of the vitamin plus normal gastric juice were high. When 11 normal subjects were tested, hepatic deposition of orally administered radioactive vitamin B₁₂ was 40 to 68 per cent of the amount noted after subcutaneous administration.

Each of the 4 patients showed neurologic improvement on vitamin B₁₂ therapy. During follow-up periods of from 2 to 96 months, neurologic symptoms did not progress.

*Boston University School of Medicine
Tufts University School of Medicine and
Boston Veterans Administration Hospital
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*Marslettsborg Hospital
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When hematologic manifestations of pernicious anemia do not occur it is difficult to differentiate combined system disease resulting from vitamin B₁₂ deficiency from other types of neurologic disorders. The authors describe a method for measuring gastrointestinal absorption of radioactive vitamin B₁₂, which they used to make this differential diagnosis in 4 patients.

Radioactive vitamin B₁₂ was given orally with and without normal human gastric juice and also subcutaneously. Scintillation from the liver was measured 10 days later. The hepatic count after subcutaneous administration was assumed to represent 100 per cent absorption. In the 4 subjects the hepatic count per minute per microcurie after oral dosage was 0 to 19 per cent of that seen after the subcutaneous dose. When the same oral dose was given with normal human gastric juice the hepatic count was 59 to 63 per cent of that obtained after injection. Gastric juice from the patients was given orally with radioactive vitamin B₁₂ to other patients with treated pernicious anemia. These patients were also given test doses of vitamin B₁₂ without gastric juice, subcutaneous doses and oral test doses with normal human gastric juice. Hepatic counts were low when the radioactive vitamin or the vitamin plus gastric juice from the patients with combined system disease was given. Counts after oral ingestion of the vitamin plus normal gastric juice were high. When 11 normal subjects were tested hepatic deposition of orally administered radioactive vitamin B₁₂ was 40 to 68 per cent of the amount noted after subcutaneous administration.

Each of the 4 patients showed neurologic improvement on vitamin B₁₂ therapy. During follow up periods of from 2 to 96 months neurologic symptoms did not progress.

*Boston University School of Medical
Dental and Veterinary School of Medicine and
Boston City Hospital
Boston, Mass.*

- 64 Arias I M, Apt L and Pollycove M. Absorption of radioactive vitamin B₁₂ in nonanemic patients with combined-system disease. *New England J Med* 253:1005-1010, Dec 8, 1955.

- 65 Holmes, J M Cerebral manifestations of vitamin B₁₂ deficiency, *Brit M J* 2 1394-1398 Dec 15, 1956

Brain lesions are a less familiar part of the neurologic syndrome of vitamin B₁₂ deficiency than lesions of the spinal cord and peripheral nerves (usually termed 'subacute combined degeneration'). However, untreated brain lesions may result in severe dementia. Evidence indicates that the mental changes are specifically related to a disturbance of cerebral metabolism which is corrected by adequate treatment with vitamin B₁₂.

Of 25 patients with vitamin B₁₂ deficiency syndrome involving the nervous system 14 showed cerebral symptoms including slow mental processes confusion and memory defect, depression delusions hallucinations optic atrophy epilepsy dysphasia agitation and manic behavior. Cerebral symptoms sometimes preceded addisonian anemia or spinal and peripheral nerve involvement by several years. Diagnosis was established by signs of spinal involvement or peripheral neuropathy, evidence of addisonian anemia in blood count or bone marrow and histamine fast achlorhydria. Doubtful cases require application of additional diagnostic methods. The author describes 2 patients who showed cerebral symptoms with normal blood and bone marrow findings. Treatment with 100 and 500 mcg of vitamin B₁₂ resulted in rapid improvement.

Spinal or cerebral manifestations require treatment with much higher doses of vitamin B₁₂ than those needed for maintenance of a normal blood count. Vitamin B₁₂ should be given in an initial dose of 1 000 mcg followed by 500 mcg twice weekly for the first month and thereafter by not less than 100 mcg weekly for the next six months. If monthly injections are given 1 000 mcg doses are advisable. Oral preparations of intrinsic factor with vitamin B₁₂ are still not fully evaluated and should not be used in the presence of neurologic symptoms.

Recent literature presents evidence that these cerebral symptoms are specifically related to vitamin B₁₂ deficiency and not to anemia.

*Birmingham Regional Hospital
Birmingham England
Staffordshire General Infirmary
Staffordshire England*

NEUROPATHIES GENERAL

- 66 Lereboullet, J and Pluvinage, R. Use of massive doses of vitamin B₁₂ in neurology (1 000 micrograms per injection), *Semaine d hôp Paris* 29 1849, June 6 1953 (abstr *J A M A* 153 601, Oct 10, 1953).

Increasing supplies of vitamin B₁₂ have made possible its use in larger doses and permitted extension of its sphere of action. The blood picture in pernicious anemia was improved more rapidly and effectively by large doses of this vitamin and some nervous lesions previously considered irreversible showed regression. Equally significant results have been obtained in other neurologic disturbances: multiple sclerosis, the polyneuritic and psychic complications of alcoholism, syndromes in the subacute superior poli-encephalitic group and essential facial neuralgia. Vitamin B₁₂ is apparently entirely nontoxic and complications resulting from its use even in doses of 1 000 mcg have not been reported.

- 67 Benbanasté J V. La vitamina B₁₂ en clinica neurológica [Vitamin B₁₂ in clinical neurology]. *Prensa méd arg* 41 1666-1668, June 11, 1954.

A patient with tabes obtained complete relief of paresthesia while receiving 10 mcg of vitamin B₁₂ twice daily but other symptoms were unaffected. Six of 7 other patients with tabes obtained rapid pain relief on 600 mcg daily and also showed objective improvement. A patient with neuroanemia receiving 30 mcg of vitamin B₁₂ daily had progressive relief of anemia and less consistent relief of

paresthesia; abnormal tendon reflexes and walking difficulties. Five patients with facial neuralgia who received 300 to 600 mcg of vitamin B₁₂ daily were considered cured after 15 days. A child with intention tremor dating from an attack of typhoid fever was still free of tremor four months after a week's course of 1 000 mcg of vitamin B₁₂ daily. Two patients with brachiocephalic neuralgia who had obtained no relief from analgesics alone improved greatly when given 400 mcg of vitamin B₁₂ in addition to the analgesics.

*Hospital Regional Español
Bahia Blanca, Argentina*

- 68 Schmidt, W. and Winter H. Vorläufige Erfahrungen mit einem hoch dosierten Vitamin B₁₂ Präparat (Dosigram 1000) [Preliminary results with a high dosage vitamin B₁₂ preparation] *Ther d Gegenw* 93:265 1954 (abstr. *Schweiz. med. Wchnschr* 85:137 Feb 5 1955).

Vitamin B₁₂ 1 000 mcg was given to 3 patients with pernicious anemia, to 3 with herpes zoster, and to 3 with neuralgia of the brachial plexus. It was effective in all these patients. It was also effective in 2 out of 4 patients with trigeminal neuralgia and in 1 of 2 with polyneuritis.

- 69 Wilson S. J., Grady H. J., Rose D. L. and Heath, H. E. A metabolic and therapeutic study of various cobalamins in multiple sclerosis. *J. Lab. & Clin. Med.* 44:954 Dec 1954 (in Soc. Proc.).

The blood of patients with multiple sclerosis has been said to contain toxic levels of cyanide, the amount being about one tenth fatal levels. Mushett and associates studied the antidotal effects of vitamin B₁₂ (hydroxocobalamin) in experimental cyanide poisoning and were able to both revive and protect their animals. These two observations logically caused us to study the metabolism and therapeutic value of cobalamins other than cyanocobalamin in patients with multiple sclerosis. All cobalamins have a marked

affinity for CN⁻ being converted instantaneously to the ordinary vitamin B₁₂ (cyanocobalamin). The molecular weights are such that 1 000 mcg of cobalamins can bind only 18 mcg of HCN.

In the present study a mixture of sulfitecobalamin and hydroxocobalamin was given intramuscularly to 13 patients with multiple sclerosis. The daily dosage was 2 000 mcg. In 3 subjects the urinary excretion of CN⁻ cyanocobalamin and cyanide-free cobalamins was determined. The method of Bover and Richards was used in determining these substances and also in preparing cyanide-free cobalamins. Base line studies revealed the following average 24 hour total urinary excretion: CN⁻, 2.0 mcg; cyanocobalamin, 30.5 mcg; and cyanide-free cobalamin, 131 mcg. During the injection of 2 000 mcg of cyanide-free cobalamins per day the following results were obtained: CN⁻, 3.6 mcg; cyanocobalamin, 231 mcg; and cyanide-free cobalamins, 714 mcg.

The patients were treated from 2 to 8 months. One patient received treatment for 16 months. Inasmuch as exacerbations of the disease occurred during this period of treatment, it was concluded that cyanide-free cobalamins have no beneficial effect and remissions observed were spontaneous. One patient was sensitive to B₁₂ and an explosive neurological involvement occurred. It was further concluded that no significant amounts of cyanide are present to convert appreciable amounts of cyanide-free cobalamins to cyanocobalamin.

Kansas City, Mo.

- 70 Levin, M. B. Vitamin B₁₂ in neuro-metabolism (preliminary clinical report). *Am J Digest Dis* 22:96-97, April 1955.

The author reviews his experience with intramuscular injections of vitamin B₁₂ in the treatment of patients with neurometabolic disorders. Dosages between 2 000 and 10 000 mcg were given two to three times weekly. Doses of 3 000 to 5 000

mcg were usually effective. Vitamin B₁₂ administered orally failed to give comparable results.

Three patients with pernicious anemia and spinal cord involvement benefited from vitamin B₁₂ and crude liver extract. In 3 patients with multiple sclerosis striking improvement in speech, swallowing, salivation, walking and nocturnal epileptiform seizures was obtained. A remission of two weeks was maintained in one patient. Improvement was also noted in patients with cerebral edema and hemorrhage with hemiplegia. These patients received the vitamin B₁₂ injections before and after the use of cortisone to absorb the clot. An infant with amyotonia improved noticeably under this regimen. After three injections a woman paralyzed by amyotrophic lateral sclerosis showed improvement. One patient with encephalomyelitis was given antibiotic therapy combined with vitamin B₁₂ and cortisone. Benefit could not be attributed to any single agent. This combination of therapy is suggested for patients with poliomyelitis. Most of the patients with radiculitis showed marked improvement within 7 to 10 days. Results were more favorable when thiamine and vitamin B₁₂ were given simultaneously.

Vitamin B₁₂ therapy was successful in treatment of retrobulbar optic neuritis, vestibular dizziness, trigeminal neuralgia, Bell's palsy and peripheral neuritis. Paroxysmal tachycardia with repeated irritation of the cardiac sympathetic nerves was reduced in 2 patients. Attacks resembling insulin shock occurred in several patients when the injections were given before breakfast.

Baltimore Md

- 71 Murphy, G. Polineuropatia alcohólica su tratamiento con vitamina B₁₂. [Alcoholic polyneuritis: treatment with vitamin B₁₂.] Prensa méd argent 41:804-806 March 19, 1954.

On the theory that alcoholic polyneuritis is a nutritional deficiency 9 pa-

tients were treated with 50 or 100 mcg of vitamin B₁₂ daily for 12 to 36 days. These 9 were chosen because of the seriousness of their symptoms and because none had received vitamins or liver extracts recently. All were men who had drunk considerable amounts daily for 6 to 30 years and often became drunk. Four were admitted with delirium tremens, 4 because of polyneuritis and 1 in a 'confused' state. Of the 9 patients, 7 obtained maximum subjective and objective relief. Pains ceased in one patient within 12 hours after the first 100 mcg dose of vitamin B₁₂. One patient was apparently unaffected by therapy even when B complex was added and one received only partial relief. The author emphasizes that these two had been alcoholics for 20 and 30 years and each had portal cirrhosis. He points out that although a similar patient obtained relief it is generally true the shorter the period of alcoholism the better the results. Symptoms in the arms were alleviated more quickly and more completely than were symptoms in the legs.

The authors conclude that vitamin B₁₂ is a therapeutic agent highly efficient in the treatment of alcoholic polyneuritis.

- 72 Bacher, K. R. Vitamin B₁₂ for treatment of alcoholic delirium. Deutsche med Wchnschr 79:1901-1903 Dec 17, 1954 (abstr J.A.M.A. 157:969 March 12, 1955).

Delirium tremens following injury or surgical trauma in 6 patients was treated with vitamin B₁₂ in intramuscular injections of 1,000 mcg. One to four injections were required in 3 patients; the number required by the others is not stated.

Delirium subsided in 5 patients (1 cachectic and gastrectomized for cancer) died. Dehydrocholic acid given intravenously to 3 of the patients before the trial of vitamin B₁₂ was ineffective.

It is recommended that vitamin B₁₂ be given prophylactically to patients running the risk of delirium tremens who

must undergo operation or receive treatment for an injury

Stuttgart Germany

DIABETIC NEUROPATHIES

- 73 Coste P D and Klinger R Use of massive doses (500 to 1 000 mcg) of vitamin B₁₂ for various conditions present in diabetic patients Policlínico (sez. prat.) 61 143 149 Feb 1, 1954 (abstr J A M A 155 781 June 19 1954)

Fifteen patients with diabetes of long standing were given from 500 to 1 000 mcg of vitamin B₁₂ daily to treat various other conditions present Five patients had lumbar and sciatic pain due to lumbosacral arthrosis 2 had neuralgia of the fifth nerve 2 had pruritus vulvae and 1 each had polyneuritis with vertebral polyarthrosis secondary brachial neuritis secondary intercostal neuritis herpes zoster diffuse psoriasis and parkinsonian tremor Diet and insulin dosage were not altered The vitamin caused no toxic effects Good results were obtained in patients with neurologic conditions In these pain was succeeded by paresthesia which disappeared as therapy was continued This was ascribed to the direct action of the vitamin on the nervous tissues Gratifying results were also observed in the 1 patient with herpes zoster and in the 2 with pruritus vulvae The vitamin had no effect however on diffuse psoriasis and parkinsonian tremor Changes in the carbohydrate metabolism attributable to the vitamin were not observed

- 74 Shuman C R and Gilpin S F Diabetic neuropathy controlled therapeutic trials Am J M Sc 227 612 617 June 1954

Thirty seven patients with diabetes mellitus had neuropathy which did not respond to adequate treatment of the diabetes Vitamin B₁₂ was administered orally or intramuscularly to 12 of these

patients in daily doses ranging from 10 to 1 000 mcg but no improvement occurred in their neurologic disorders Pregnant mammalian liver adenosine triphosphate with thiamine and pantothenic acid were also ineffective in producing objective improvement

Temple University Hospital and School of Medicine Philadelphia Pa

- 75 Sauer H and Dussler A Disease aspect of diabetic polyneuritis and its treatment with vitamin B₁₂ Deutsche med Wchnschr 79 1046-1048 June 25 1954 (abstr J A M A 156 643, Oct 9 1954)

Diabetic polyneuritis in 18 patients was treated with vitamin B₁₂ given intramuscularly or subcutaneously The dosage was usually 30 to 60 mcg daily for two weeks a few patients received 1 000 mcg daily Fifteen patients improved and 3 (2 of them despite high dosage) did not

Stuttgart Germany

- 76 Müller D S and Lichtman W F Diabetic neuropathic arthropathy of feet Summary report of seventeen cases A M A Arch Surg 70 513 518 April 1955

The increased incidence of foot deformities accompanying diabetes is pointed out Such arthropathies usually occur in poorly controlled long standing diabetes and are due to involvement of the autonomic nervous system rather than to poor circulation Out of 17 of the authors patients with this complication 16 had refused or neglected treatment of the diabetes The usual symptoms and signs were reduced vibratory and position sense diminished reflexes and a widened shortened everted foot often accompanied by bone displacement or destruction Recommended treatment includes better control of the diabetes good foot hygiene and if necessary amputation or sympathectomy Supportive treatment consists of administration of vitamins B₁ and B₁₂

dilute hydrochloric acid, crude liver extract and injections of pregnant marmoset liver extract. Vasodilators and antibiotics may also be indicated.

*Cook County Hospital and
Chicago Medical School
Chicago III*

- 77 Lueck, A. G. Neurologic disturbances complicating diabetes. *J Iowa Med Soc* 46:296-300, June 1956

In discussing the neurologic disturbances complicating diabetes, the author divides the disturbances into two groups: peripheral neuritis and autonomic neuropathy and presents 18 cases. The diabetes was brought under control and in 16 patients injections of vitamin B₁₂ were added to the regimen. In most cases pain either subsided or disappeared. In one case strabismus disappeared after two months of therapy.

The author states that this therapy is of questionable value but it is my impression that for the present it should be used. Practically all diabetics with neuropathy are taking insulin or should be and they can easily inject vitamin B₁₂ once a day for themselves without great cost. Beyond these measures nothing on the horizon holds any promise of significance.

Des Moines, Iowa

TRIGEMINAL NEURALGIA

- 78 Alexander II, Jr. and Davis, C. H., Jr. Trigeminal neuralgia: conservative management with massive vitamin B₁₂ therapy. *North Carolina M J* 14:206-207, May 1953 (abstr. *Clin Med* 61:400, May 1954)

The apparent effectiveness of small doses of vitamin B₁₂ in relieving the neuropathy of pernicious anemia and in the treatment of certain peripheral neuritis suggested its use in trigeminal neuralgia. Accordingly 17 patients with trigeminal neuralgia have been treated with

100 mcg of vitamin B₁₂ given intramuscularly over a 10-day period. Of these 6 had complete relief lasting from 2 to 8 months and 2 had satisfactory relief. In 4 patients previously treated with surgery or alcohol nerve blocks, pain relief was delayed but was eventually satisfactory. The results warrant continued investigation of this treatment for trigeminal neuralgia.

- 79 Surtees, S. J. and Hughes, R. R. Treatment of trigeminal neuralgia with vitamin B₁₂. *Lancet* 1:439-441, Feb. 27 1954

Vitamin B₁₂ administered to 18 patients with trigeminal neuralgia was followed by considerable improvement or complete relief of pain in 14, little or no immediate improvement in 3 and moderate improvement in 1. A patient with glossopharyngeal neuralgia also received marked relief after vitamin B₁₂ therapy. Some patients received 1,000 mcg of vitamin B₁₂ intramuscularly twice weekly for five doses, some 1,000 mcg daily for 10 days followed by 1,000 mcg twice weekly for five doses, and a few 1,000 mcg twice daily for periods up to three weeks.

Injections of 1,000 mcg of vitamin B₁₂ administered twice weekly for five doses failed to relieve the pain of 3 patients with postherpetic neuralgia and of 2 with facial neuralgia precipitated by operative procedures. The authors emphasize that failure may have been caused by inadequate dosage.

*Royal Southern Hospital
Liverpool, England*

- 80 Albrecht, K. and Krump, J. Diagnose Differentialdiagnose und Behandlungsmöglichkeiten der Trigeminusneuralgie (Unter besonderer Berücksichtigung der konservativen Behandlung mit Hydantoinderivaten und Vitamin B₁₂). [Diagnosis, differential diagnosis and therapeutic possibilities in trigeminal neuralgia: conservative treatment with hydan-

toin drugs and vitamin B₁₂] München med Wchnschr 96 1037 1039, Sept 3 1954

Vitamin B₁₂ was given in daily intramuscular doses of 1 000 mcg for 10 days to 13 patients with trigeminal neuralgia. Improvement began after about the fourth injection. Of the 13 patients 7 (including 2 whose neuralgia followed herpes zoster) were completely relieved. 1 with long standing neuralgia was so much improved that she refused surgery. 1 patient was not benefited. No comment is made on the results in the remaining patients although early in the article the authors state that vitamin B₁₂ was used with good effect in trigeminal neuralgia.

Erlangen, Germany

HERPES ZOSTER

- 81 Schiller F Herpes zoster Review with preliminary report on new method for treatment of postherpetic neuralgia J Am Geriatrics Soc 2 726-735 Nov 1954

Pathogenesis and diagnosis of herpes zoster are discussed with emphasis on the treatment of postherpetic neuralgia. Among the many treatments tried vitamin B₁₂ is reported to have some merit. The author has observed 1 patient with relatively mild postherpetic pain who derived some benefit. Its use as an anti-neuralgic agent is recommended on the basis that it may improve the metabolism of nerve cells which are not irreversibly damaged. It is also noted that in large doses (1 000 mcg once or twice weekly) vitamin B₁₂ induces a certain well being in the elderly.

The author's proposed method of treatment of neuralgic pain which has been used with some benefit in 10 patients: ■ segmental counterirritation injection

of small amounts of hypertonic saline into paravertebral muscle

University of California
Medical School
San Francisco Calif

- 82 Jolles K E Herpes zoster treated with vitamin B₁₂ Brit M J 1 166 167, Jan 15 1955 (In Correspondence)

The writer comments further on his successful treatment of herpes zoster with three or four intramuscular doses of 50 mcg of vitamin B₁₂. Doubling the dose did not produce more beneficial effects. Oral treatment (150 mcg four times daily) was also effective within eight days in one elderly woman with herpes zoster ophthalmicus.

West Bromwich England

- Treatment of herpes zoster J A M A 163 482 Feb 9 1957 (in Foreign Letters India)

J R Srivastava gave vitamin B₁₂ to 10 patients with herpes zoster (J Indian M A 27 10 1956). There was marked relief from pain, rapid disappearance of vesicles and crusts and no postherpetic pain. The results on the whole were excellent.

- 84 Wigoder R G B and Jeffs H G Treatment of herpes zoster Brit M J 1 523 March 2 1957 (in Correspondence)

The authors treated 21 patients with herpes zoster with daily 100 mcg injections of vitamin B₁₂ for six days. Eruptions were treated with flexible collodion twice daily.

Pain ceased towards the end of the treatment period in all but 2 patients in whom it was especially severe and persisted for three months. In these cases one 1 000 mcg injection was given on the first and third days and three 250 mcg injections on three alternate days.

GASTROINTESTINAL

GASTRECTOMY

- 85 Ley, A E and Sharpe L E The absorption of vitamin B₁₂ after total gastrectomy, *Clin Research Proc* 2 31, March 1954

In a study of the absorption of vitamin B₁₂ after total gastrectomy, Co⁵⁷ vitamin B₁₂ 0.5 to 1.0 mcg, was given to 6 totally gastrectomized patients to 1 patient with pernicious anemia, and to 2 normal persons. Stools were subsequently examined for radioactivity. Normal subjects absorbed approximately 65 per cent of the dose. The patient with pernicious anemia, in early remission, absorbed approximately 22 per cent, when the same dose was administered with intrinsic factor the absorption increased to 49 per cent. Gastrectomized patients absorbed from 0 to 22 per cent with intrinsic factor their absorption varied from 36 to 59 per cent.

One patient gastrectomized 5 years before this study had a mild macrocytic anemia with a hematocrit of 35 per cent. The bone marrow aspirate on this patient showed equivocal megaloblastosis. The patient was treated with vitamin B₁₂ 5 mcg by mouth each day. During the ensuing seven weeks the hematocrit fell to 32.8 per cent. Treatment for the next eight weeks with the same daily dose of vitamin B₁₂ in addition to an intrinsic factor concentrate was followed by a rise in the hematocrit to 43 per cent with the mean corpuscular volume decreasing to nearly normal. The authors believe these findings demonstrate the importance of the gastric mucosa in the absorption of vitamin B₁₂.

*Memorial Center for Cancer and Allied Diseases
New York N Y*

- 86 McCorkle, H J and Harper H A The problem of nutrition following complete gastrectomy, *Ann. Surg* 140 467-474 Oct 1954

A review of the nutritional status of 24 totally gastrectomized patients indicated that only 6 had gained significant amounts of weight postoperatively. Only 9 could eat comfortably.

To improve digestion and nutrition following total gastrectomy a study was made of 120 dogs subjected to the operation. The study showed that alimentary time was decreased although absorption of amino acids was more rapid in gastrectomized animals than in controls. Increased nitrogen loss in the stool showed that it was less efficient. Most of the dogs remained in positive nitrogen balance however, provided they ate enough protein. Rates of absorption of fat and of glucose were increased. Severe postoperative diarrhea was prevented by giving 3 Gm of neomycin daily for two days once each month and a few drops of hydrochloric acid daily. Anemia occurred after gastrectomy but could be minimized by vitamin therapy. The dogs were given intrinsic factor and 10 mcg of vitamin B₁₂ every third day but the authors believe that larger oral doses of vitamin B₁₂ might have prevented anemia even without intrinsic factor.

The results indicate that good nutrition and weight can be maintained after total gastrectomy if isoperistaltic continuity between the esophagus and duodenum is achieved and if the animals can be trained to eat slowly and often.

Three patients attained good nutritional status after gastrectomy and anastomosis of an isoperistaltic segment of colon between the esophagus and duodenum had adequate food capacity.

and appetite and digestive comfort The importance of giving large oral doses of vitamin B₁₂ to patients who had undergone total gastrectomy is emphasized

*University of California
School of Medicine
San Francisco Calif*

posium # 384-410 1954 (abstr Blood 11 96 Jan 1956)

Pernicious anemia occurred in all of 4 patients observed for more than 5 years after total gastrectomy Development of anemia after operation was as follows hypochromic or orthochromic anemia in the 1st or 2nd year hyperchromic anemia in the 3rd pernicious anemia in the 5th or 6th Folic acid concentration in the blood dropped during the early stage more than 3 years after operation not only folic acid but also vitamin B₁₂ was decreased In the reported 4 cases anemia was treated successfully by folic acid or vitamin B₁₂ Relapse occurred after interruption of treatment

*Kyushu University School of Medicine
Fukuoka Japan*

- 87 Paulson M and Harvey J C Hematological alterations after total gastrectomy Evolutionary sequences over a decade JAMA 156 1556-1560 Dec 25 1954

Hematologic study of 27 totally gastrectomized (for carcinoma of the stomach) patients revealed that an iron deficiency anemia developed shortly after operation then within two years macrocytosis developed in the peripheral blood and if survival was long enough a true macrocytic megaloblastic anemia developed The iron deficiency could be controlled by routine administration of ferrous gluconate or ferrous sulfate The macrocytic megaloblastic anemia was responsive to parenteral vitamin B₁₂ Treatment given to 5 patients is shown in a table Initially 4 of these patients were given weekly intramuscular injections of vitamin B₁₂ ranging from 30 to 150 mcg for about seven months followed by the same dose once a month for 6 6 26 and 24 months respectively Two of these patients also received oral folic acid 15 mg daily Oral vitamin B₁₂ was also tried (unsuccessfully) in one of the 4 patients The fifth patient was treated unsuccessfully with folic acid but later he responded to crude liver

A study of intestinal absorption of vitamin B₁₂ was made in one gastrectomized patient Co⁶⁰ vitamin B₁₂ 0.5 mcg was given orally About 17 per cent of the radioactive vitamin was recovered in the stool (normal recovery 10 to 40 per cent)

*Johns Hopkins University and Hospital
Baltimore Md*

- 89 Mauri Paolini A and Marinoni E Hematologic changes following total gastrectomy Chirurgia 10 331 1955 (Internat Abstr Surg 103 359 1956 in Surg Gynec & Obst Oct 1956)

After a careful review of many reports of hyperchromic megaloblastic anemia following total gastrectomy the authors conclude that development of this anemia is directly related to the operation Most cases occur 5 or 6 years after gastrectomy and patients respond well to treatment with vitamin B₁₂ and liver extract The authors recommend parenteral administration

- 90 Pitney W R and Beard M F Vitamin B₁₂ deficiency following total gastrectomy AMA Arch Int Med 95 591 593 April 1955

Lack of intrinsic factor causes decreased absorption of vitamin B₁₂ after total gastrectomy but megaloblastic anemia usually does not result until at least two years after operation because the body possesses a considerable reserve of vitamin B₁₂ As this is gradually depleted serum levels of vitamin B₁₂ fall thus measurement of these levels after opera-

- 88 Tomoda M Agastric pernicious anemia Rept of Hematological Sym

GASTROINTESTINAL

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New York N Y*

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*University of California
School of Medicine
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Johns Hopkins University and Baltimore Md

- 88 Tomoda M Agastric pernicious anemia Rept of Hematological Sym

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After a careful review of many reports of hyperchromic megaloblastic anemia following total gastrectomy the authors conclude that development of this anemia is directly related to the operation Most cases occur 5 or more years after gastrectomy and patients respond well to treatment with vitamin B₁₂ and liver extract The authors recommend parenteral administration

- 90 Pitney W R and Beard M F Vitamin B₁₂ deficiency following total gastrectomy A M A Arch Int Med 95 591 593 April 1955

Lack of intrinsic factor causes decreased absorption of vitamin B₁₂ after total gastrectomy but megaloblastic anemia usually does not result until at least two years after operation because the body possesses a considerable reserve of vitamin B₁₂ As this is gradually depleted serum levels of vitamin B₁₂ fall thus measurement of these levels after opera

tion provides an accurate index of the state of vitamin B₁₂ nutrition

One 57 year old woman gastrectomized for carcinoma did not develop megaloblastic anemia but her serum concentrations of vitamin B₁₂ progressively fell from over 150 micromicrograms per cc 4 months after operation to less than 50 micromicrograms about 18 months after operation (when she died of metastases) The Schilling test showed that she absorbed normal amounts of orally administered vitamin B₁₂ only when it was given with normal gastric juice

In view of these findings the authors recommend administration of vitamin B₁₂ to gastrectomized patients even when blood cell count is normal Thus will not only prevent development of megaloblastic anemia but will also prevent other manifestations of vitamin B₁₂ deficiency such as malaise anorexia and lassitude which might be mistaken for signs of metastasized carcinoma in some cases

- 91 Badenoch, J Evans J R Richards W C D and Witts L J Megaloblastic anemia following partial gastrectomy and gastro enterostomy, Brit J Haemat 1 339, 344 Oct 1955 (abstr J A M A 160 321-322, Jan 28 1956)

Although megaloblastic anemia due to deficiency in vitamin B₁₂ is almost inevitable after total gastrectomy if the patient survives the operation for six years or longer, it is rare after operations in which a portion of the stomach remains Recently the authors observed five patients in whom megaloblastic anemia developed after partial gastrectomy and one patient in whom it developed after gastro enterostomy Since intrinsic factor is secreted by the body of the stomach it seemed unlikely that the megaloblastic anemia following partial gastrectomy could be due to lack of this factor However new techniques of investigation made it possible to study such anemias more fully All the patients had a macrocytic

anemia with megaloblasts in the sternal marrow They were treated with vitamin B₁₂ and four showed optimal response A fifth showed clinical improvement but although the bone marrow changed from megaloblastic to normoblastic erythropoiesis there was no significant alteration in the peripheral blood a course of folic acid was ineffective but treatment of the accompanying iron deficiency resulted in a good response The sixth patient whose anemia was mild showed no therapeutic response to either vitamin B₁₂ or folic acid The interval between operation and the diagnosis of megaloblastic anemia ranged from 2 to 17 years In all instances the total duration of dyspepsia before and after operation had been 20 years or longer The authors investigated the excretion of acid and uropepsin, and the absorption and excretion of fat They also attempted to obtain biopsy specimens from the remaining portion of the stomach and estimated the vitamin B₁₂ content of the serum and the secretion of intrinsic factor The results of the studies indicated that in five of the subjects the anemia was caused by vitamin B₁₂ deficiency There was failure to absorb vitamin B₁₂ which was corrected by the addition of intrinsic factor the biopsy studies revealed atrophy of the gastric body mucosa comparable in severity to that seen in pernicious anemia In the sixth patient the nature of the deficiency and its pathogenesis remained obscure Steatorrhea was not a significant factor in any of the cases Since megaloblastic anemia is rare after partial gastrectomy and gastroenterostomy it seemed possible that the patients might have Addisonian pernicious anemia and that the gastric operation was only incidental This hypothesis was not tenable in view of the absence of a family history of pernicious anemia and because the histological study of the stomach removed at partial gastrectomy showed normal gastric body glands and in no way resembled the mucosal atrophy seen in pernicious anemia The biopsy

studies did not allow a detailed comparison between the stomach in this condition and in idiopathic pernicious anemia but the authors gained the impression that gastritis was present and they feel that chronic gastritis associated with prolonged dyspepsia gastric ulceration and operative intervention may have contributed to the development of gastric atrophy

- 92 MacLean L. D. The differentiation of achylia gastrica and achlorhydria by means of radioactive vitamin B₁₂. *Gastroenterology* 29 653 665 Oct 1955

The vitamin B₁₂ excretion test differentiates patients with simple achlorhydria and achylia gastrica. The latter are possibly more likely to develop gastric cancer. Pernicious anemia patients develop gastric cancer more commonly than those who do not have pernicious anemia.

This study showed that gastrectomized patients lack the intrinsic factor necessary for absorption of oral vitamin B₁₂. A follow up study of patients who survived for over three years after total gastrectomy without vitamin B₁₂ or liver extract therapy has shown that 90 per cent develop megaloblastic marrow. Apparently pernicious anemia is a late but an inevitable sequela of total or proximal subtotal gastrectomy but is not likely to occur until at least 3 years after the operation.

*University of Minnesota
Minneapolis, Minn*

- 93 MacLean L. D. and Sundberg R. D. Incidence of megaloblastic anemia after total gastrectomy. *New England J Med* 254 885-893 May 10 1956

The authors review of 17 cases of megaloblastic anemia following total gastrectomy reported in the literature suggests that if the patient survives long enough megaloblastic anemia will occur.

The authors state that 10 of 11 totally gastrectomized patients (observed by them) who did not receive prophylactic

therapy for pernicious anemia and who survived for over three years developed a megaloblastic bone marrow. Megaloblastosis occurred irrespective of the type of enteric reconstruction and was not due to blind loop anemia.

Six patients with total gastrectomy and 3 with proximal subtotal gastrectomy were unable to absorb oral Co⁵⁷ vitamin B₁₂ until intrinsic factor was added. This was evidence that the site of intrinsic factor production is the body and fundus the area of pathologic change in the stomach in pernicious anemia. The human antrum apparently is not important in elaboration of intrinsic factor.

Vitamin B₁₂ or purified liver extract alone produced remissions in these patients. The authors state that all patients subjected to total gastrectomy or proximal subtotal gastrectomy with esophago-anastomosis should receive continuous prophylactic parenteral vitamin B₁₂ or liver extract therapy commencing within three years after operation.

*University of Minnesota Medical School
Minneapolis, Minn*

- 94 Harvey J. C. The vitamin B₁₂ deficiency state engendered by total gastrectomy. *Surgery* 40 977 989 Nov 1956 (in *Recent Advances in Surgery*)

In 30 patients treatment with liver extracts or vitamin B₁₂ was purposely withheld after total gastrectomy in order to observe the development of vitamin B₁₂ deficiency. Of the 30 patients 10 were alive at the time of writing their post operative survival time ranging between 2½ years and 17 years.

The first evidence of vitamin B₁₂ deficiency was macrocytosis of erythrocytes which occurred from six months to seven years (average two and one half years) after gastrectomy. Anemia appeared next within an average of four years postoperatively and megaloblasts invariably were found in the marrow within one year after that. Three patients who failed to come in for observation according to in

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anemia with megaloblasts in the sternal marrow. They were treated with vitamin B₁₂ and four showed optimal response. A fifth showed clinical improvement but, although the bone marrow changed from megaloblastic to normoblastic erythropoiesis there was no significant alteration in the peripheral blood. A course of folic acid was ineffective but treatment of the accompanying iron deficiency resulted in a good response. The sixth patient, whose anemia was mild, showed no therapeutic response to either vitamin B₁₂ or folic acid. The interval between operation and the diagnosis of megaloblastic anemia ranged from 2 to 17 years. In all instances the total duration of dyspepsia before and after operation had been 20 years or longer. The authors investigated the secretion of acid and uropepsin, and the absorption and excretion of fat. They also attempted to obtain biopsy specimens from the remaining portion of the stomach and estimated the vitamin B₁₂ content of the serum and the secretion of intrinsic factor. The results of the studies indicated that in five of the subjects the anemia was caused by vitamin B₁₂ deficiency. There was failure to absorb vitamin B₁₂ which was corrected by the addition of intrinsic factor. The biopsy studies revealed atrophy of the gastric body mucosa comparable in severity to that seen in pernicious anemia. In the sixth patient the nature of the deficiency and its pathogenesis remained obscure. Steatorrhea was not a significant factor in any of the cases. Since megaloblastic anemia is rare after partial gastrectomy and gastroenterostomy it seemed possible that the patients might have Addisonian pernicious anemia and that the gastric operation was only incidental. This hypothesis was not tenable in view of the absence of a family history of pernicious anemia and because the histological study of the stomach removed at partial gastrectomy showed normal gastric body glands and in no way resembled the mucosal atrophy seen in pernicious anemia. The biopsy

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University of Minnesota Medical School
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The first evidence of vitamin B₁₂ deficiency was macrocytosis of erythrocytes which occurred from six months to seven years (average two and one half years) after gastrectomy Anemia appeared next within an average of four years postoperatively and megaloblasts invariably were found in the marrow within one year after that Three patients who failed to come in for observation according to in

structions had combined nervous system disease six seven and eight years respectively after operation

Parenteral vitamin B₁₂ therapy was given to 12 patients whose hematologic picture indicated vitamin B₁₂ deficiency. All these patients had good hematologic and neurologic responses. The author recommends that every totally gastrectomized patient be given parenteral vitamin B₁₂ regularly.

*Johns Hopkins University and Hospital
Baltimore Md*

- 95 Halsted J A, Briggs J D and Gasster M. Nutritional problems after total gastrectomy, *New York State J Med* 57 223-231, Jan 15, 1957

Of 52 totally gastrectomized patients 34 died (of recurrent carcinoma) and 18 survived of whom 17 were available for study six months to nine years post operatively.

Of the 17 patients 12 showed evidence of mild hypochromic anemia due to iron deficiency. Since none showed evidence of blood loss it is presumed that an iron absorption defect may exist after gastrectomy and that anemic patients may have lost considerable blood before operation. Ferrous sulfate given to 4 patients produced a rise in hemoglobin.

Of 9 patients who received no prophylactic therapy megaloblastic anemia due to vitamin B₁₂ deficiency developed in only 1 becoming marked five years after operation. Therapy with parenteral vitamin B₁₂ produced an excellent hematologic response. Megaloblastic anemia did not develop in the 7 patients receiving prophylactic vitamin B₁₂, liver extract or folic acid therapy.

In 12 patients undergoing radioactive vitamin B₁₂ absorption tests 80 to 100 per cent of the orally administered radioactivity was recovered indicating little or no absorption of the vitamin. When the test dose was mixed with 50 cc of nor-

mal human gastric juice most of the Co⁶⁰ vitamin B₁₂ was absorbed and in 7 patients absorption was greater than normal. The authors conclude that the site of origin of intrinsic factor in man is limited to the stomach.

The small incidence of pernicious anemia following total gastrectomy is explained as follows: (1) the daily requirement of vitamin B₁₂ is small (1 mcg) and the liver contains approximately a three year supply. Thus megaloblastic anemia rarely occurs until at least three years after operation and few patients survive that long. In some cases a section of stomach which secretes intrinsic factor is not removed; in others, liver extract or vitamin B₁₂ is administered.

The authors state: Patients who have had a total gastrectomy should receive vitamin B₁₂ indefinitely to prevent the development of B₁₂ deficiency with megaloblastic anemia. It must be given parenterally since it cannot be absorbed after removal of the stomach. However oral preparations containing intrinsic factor concentrates with vitamin B₁₂ should also be effective. An appropriate schedule of parenteral administration is 30 to 50 mcograms once a month.

*University of California Medical School and
Veterans Administration Hospital
Los Angeles Calif*

INTESTINAL ANOMALIES

- 96 Pearson R D. Macrocytic anemia associated with intestinal strictures and anastomoses. *Ann Int Med* 40 600-610 March 1954

Case histories of 2 patients with macrocytic anemia associated with intestinal strictures and anastomoses are presented. One showed a submaximal response to folic acid and then to intramuscular vitamin B₁₂; the other responded well to intramuscular vitamin B₁₂. The mechanism by which intestinal strictures or anastomoses cause macrocytic anemia is un-

known Removal or short circuiting of considerable lengths of small intestine may result in inadequate absorption of hemopoietic substances or stagnation of intestinal contents may cause changes in bacterial flora which interfere with formation or utilization of hemopoietic substances

*Y Terrazs Adm Ist ation Hospit I
B onk N Y*

- 97 Doscherholmen A and Hagen P S Absorption of Co⁵⁷ labeled vitamin B₁₂ in intestinal blind loop megaloblastic anemia J Lab & Clin Med 44 790 Nov 1954 (in Soc. Proc)

A laborer was admitted to the hospital in 1952 with a history of progressive weakness intermittent glossitis and numbness of the hands and feet of 6 months duration In 1944 an ileocolostomy for terminal ileitis had been followed by a second laparotomy with closure of the loop of ileum distal to the anastomosis Gradual recovery ensued although he had 4 to 6 loose stools daily On admission blood examination revealed a macrocytic anemia and a megaloblastic bone marrow Gastric juice contained free hydrochloric acid Fecal fat and nitrogen studies were normal X ray examination revealed an ileocolostomy with a blind loop of distal ileum and evidence of ileitis Vitamin C gave suboptimal response while all blood values returned to and remained normal with continued administration of vitamin B₁₂ intramuscularly In 1954 physical and laboratory findings were normal but x ray examination revealed progression of the ileitis Absorption studies with 0.5 mcg Co⁵⁷ vitamin B₁₂ given orally resulted in fecal excretion of 78 per cent no urinary radioactivity followed a flushing intramuscular dose of 1000 mcg of vitamin B₁₂

The addition of up to 300 mg of intrinsic factor concentrate pretest saturation with oral vitamin B₁₂ and attempted

sterilization of the gut with neomycin failed to cause normal vitamin B₁₂ absorption The patient's gastric juice however effected some absorption of Co⁵⁷ vitamin B₁₂ in a patient with pernicious anemia

Min capelle Minn

- 98 Badenoch J Bedford P D and Evans J R Massive diverticulosis of the small intestine with steatorrhea and megaloblastic anemia Quart J Med 24 321 330 1955 (abstr Blood 11 1053 1054 Nov 1956)

Three cases of steatorrhea with megaloblastic anemia have now been described in association with massive diverticulosis of the small intestine Additional observations are reported on one of these cases and three others are described In one of the four cases diverticulosis was not suspected but was found post mortem in the others the condition was demonstrated by barium meals In two the absorption of labeled cyanocobalamin was investigated and found to be grossly impaired but this defect was not completely corrected by the addition of the small amount of intrinsic factor which is effective in pernicious anemia A further improvement occurred when larger amounts of intrinsic factor were given as occurs in those cases of idiopathic steatorrhea where megaloblastic anemia is associated with malabsorption of vitamin B₁₂ It is considered that jejunal diverticulosis causes steatorrhea and that the megaloblastic anemia is secondary to this Unlike what occurred in one case of pernicious anemia and one of idiopathic steatorrhea the administration of Aureomycin enhanced the absorption of labeled cyanocobalamin in the two patients with intestinal diverticulosis and megaloblastic anemia who were investigated in this way

*Radcliffe I firma y nd
Co 1 y Road Hospital
Oxfo d E gla d*

- 99 Thompson, R B and Ungley, C C
Megaloblastic anemia associated with
anatomic lesions in the small intestine, Blood 10 771 787, Aug 1955

Authors summary 'This paper describes the development of anemia in six patients with strictures and anastomoses in the small intestine. The marrow proved to be megaloblastic in three instances and megaloblastic change is presumed in the other three because of the clinical and laboratory findings, and the characteristic hemopoietic response to liver therapy. Responses to crude and refined liver extracts [5 cc] and vitamin B₁₂ [80 mcg orally and 80 to 160 mcg intramuscularly] compared unfavorably with those to be expected in Addisonian pernicious anemia; they were similar to the poorer responses often observed in megaloblastic anemia associated with idiopathic steatorrhea. The relationship of the intestinal lesion to the development of megaloblastic anemia is briefly discussed.

Royal Victoria Infirmary
Newcastle on Tyne, England

- 100 Halsted, J A, Lewis, P M and Gasster, M. Absorption of radioactive vitamin B₁₂ in the syndrome of megaloblastic anemia associated with intestinal stricture or anastomosis, Am J Med 20 42 52, Jan 1956

The effect of antibiotics and intrinsic factor on the absorption of vitamin B₁₂ was studied in 2 patients with megaloblastic anemia associated with intestinal stricture or anastomosis. Both patients responded to parenteral vitamin B₁₂ but developed anemia when it was discontinued. Test doses of 0.5 mcg of Co⁵⁷ vitamin B₁₂ were given alone then with intrinsic factor and later immediately following daily administration of 2 Gm of Aureomycin or chloramphenicol or 6 Gm of neomycin and again after 10 to 14 days.

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patient. Both Aureomycin and Chloramphenicol produced markedly increased absorption of vitamin B₁₂ but neomycin had no effect. Since Chloramphenicol and Aureomycin (but not neomycin) also decreased diarrhea in 1 patient, it was thought that the increased absorption of vitamin B₁₂ might be due to this effect rather than to the direct effects of the antibiotics. A test was therefore done using Gantresin which completely cured the diarrhea but caused no decrease in fecal excretion of vitamin B₁₂. Thus diarrhea was not the reason for the high excretion of vitamin B₁₂ in this patient.

The authors in their summary state

These studies provide additional support for the concept that abnormal bacterial growth in the small intestine may result in impaired utilization of vitamin B₁₂ with the development of megaloblastic anemia in some instances. The exact mechanism whereby intestinal bacteria may affect hematopoiesis adversely and certain antibiotics may favorably influence it, is not yet clear.

University of California Medical Center
Los Angeles, Calif

SPRUE AND STEATORRHEA

- 101 Glass, G B J. Intestinal absorption and hepatic uptake of vitamin B₁₂ in diseases of the gastrointestinal tract, Gastroenterology 30 37 52 Jan 1956

Absorption of vitamin B₁₂ was studied by giving patients an oral tracer dose of 0.5 mCi of Co⁵⁷ vitamin B₁₂ and measuring

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min B₁₂ in 10 patients with intestinal disease. The absorption in 4 patients with irritable colon was good. In 4 with sprue and in 2 with regional ileitis no hepatic uptake of oral vitamin B₁₂ occurred and this could not be corrected by giving intrinsic factor.

The author states that in the future determination of the intestinal absorption of vitamin B₁₂ by the isotope technique probably will permit determination of the extent of gastric atrophy. This would be helpful in detecting the precursors of pernicious anemia among the apparently healthy persons and would aid in differentiating pernicious anemia from other macrocytic anemias. The test also permits the detection of a sprue-like absorption pattern.

New York Medical College and
Flower and Fifth Avenue Hospitals
New York, N. Y.

- 102 Meynell M J, Cooke W T, Cox E V and Gaddie R. Serum-cyanocobalamin level in chronic intestinal disorders. *Lancet* 1 901 904 May 4 1957

A method is presented for the assay of vitamin B₁₂ in sera using *L. leichmannii*. Serum levels were studied in 78 patients with idiopathic steatorrhea, in 43 with regional ileitis, in 90 control hospital patients with various diseases and in 43 healthy volunteers.

Ninety six per cent of all controls had levels within the normal range (100 to 450 micromicrograms per cc). The 2 control patients with levels below normal had peptic ulcers. Of 3 controls with levels above normal, 1 had congestive cardiac failure and hepatomegaly.

In 22 of 78 patients with idiopathic steatorrhea the serum cyanocobalamin levels were below 100 micromicrograms per cc and in 14 others a deficiency was diagnosed from the hematologic response to vitamin B₁₂ therapy.

Of 43 patients with regional ileitis 23 had abnormally low serum cyanocobalamin levels and 3 others were being treated

with cyanocobalamin for megaloblastic anemia. Most patients with regional ileitis had free hydrochloric acid in the stomach but some had loss of weight, energy and concentration, irritability, personality changes and occasional psychoses which cleared during vitamin B₁₂ therapy. Most patients with steatorrhea or ileitis had no hematologic manifestations.

The serum cyanocobalamin levels in idiopathic steatorrhea and in regional ileitis contrast with the normal levels found in enterocolitis, in ulcerative colitis and even more strikingly with the high levels (280 to 1 250 micromicrograms per cc) found in cirrhosis.

General Hospital
Birmingham, England

- 103 Oxenhorn S, Estren S and Adlersberg D. Intestinal uptake of vitamin B₁₂ in the malabsorption syndrome. *J Mt Sinai Hosp* 24 232 242 May June 1957

A modification of Schilling's urinary excretion test was employed in the study of vitamin B₁₂ absorption in pernicious anemia and the malabsorption syndrome. Severely defective vitamin B₁₂ absorption was noted in all patients with pernicious anemia. This defect was fully corrected by the addition of intrinsic factor. Deficient vitamin B₁₂ absorption was also noted in 20 of 25 patients with idiopathic sprue and in 3 with a sprue syndrome secondary to the surgical removal of most of the small intestine. Intrinsic factor failed to improve vitamin B₁₂ absorption in either of these groups.

The Mount Sinai Hospital
New York, N. Y.

- 104 Estren S. The blood and bone marrow in idiopathic sprue. *J Mt Sinai Hosp* 24 304-316 May June 1957

There are multiple intestinal defects of absorption in idiopathic sprue. Those of importance in the development of anemia include malabsorption of dietary protein, folic acid, vitamin B₁₂ and iron. Re-

- 99 Thompson, R H and Ungley, C C
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scintillation counting of the liver area.

Impaired absorption of vitamin B₁₂
was found in some but not all patients
with gastric anacidity in elderly patients
with gastric hypoacidity or anacidity and
in partially gastrectomized patients. Ab-
sorption levels in all these patients were
increased by concomitant administration
of intrinsic factor.

Measurement of hepatic uptake was
also used to study absorption of oral vita-

vitamin B₁₂ to a specific portion of gastric juice by paper electrophoresis may be a clue to the further identification of the intrinsic factor of Castle
University of Wisconsin Medical School
Madison, Wis

109 Council on Pharmacy and Chemistry
Vitamin B₁₂ with intrinsic factor concentrate JAMA 153 463 Oct 10 1953

Vitamin B₁₂ also known as the intrinsic factor when combined with intrinsic factor concentrate is effective orally for the treatment of pernicious anemia with and without neurological complications. It is also effective by virtue of its vitamin B₁₂ content in the treatment of tropical and nontropical sprue nutritional macrocytic anemia caused by vitamin B₁₂ deficiency and macrocytic anemia of infancy.

Oral vitamin B₁₂ with intrinsic factor concentrate is suitable to replace injectable cyanocobalamin or liver for [pernicious anemia] patients in whom parenteral therapy is difficult or undesirable. Patients should be carefully observed during oral treatment if expected improvement does not occur further examination should be made to rule out complicating disorders such as infection gastrointestinal malfunction or undiagnosed malignant disease. In the presence of any such complication the dosage may need to be increased or abandoned in favor of injection therapy with cyanocobalamin or liver.

The potency is expressed in terms of the U.S.P. oral unit of hematopoietic activity. The average daily dosage for the treatment of pernicious and related macrocytic anemias is 1 U.S.P. oral unit daily.

110 Schilling R.F. A new test for intrinsic factor activity J Lab & Clin Med 42 946-947 Dec 1953 (in Soc Proc)

Progress toward the isolation of intrinsic factor has been hampered by the necessity of a tedious bioassay in patients

with pernicious anemia in relapse. As part of a new test for intrinsic factor activity 6 normal persons and 10 pernicious anemia patients in remission were each given 2 mcg of radioactive vitamin B₁₂ orally and two hours later 1 000 mcg of nonradioactive vitamin B₁₂ by subcutaneous injection. Within four to six hours after the oral dose radioactivity appeared in the urine of the normals persisting for at least 20 hours but it did not appear in urine of the pernicious anemia patients. When the test was repeated in pernicious anemia patients with addition of human gastric juice to the radioactive vitamin B₁₂ they all excreted radioactivity in the urine. Boiling the gastric juice destroyed its ability to produce this effect as did giving it 12 hours after the oral Co⁶⁰ vitamin B₁₂ dose. Two preparations of hog duodenum with intrinsic factor activity also produced radioactivity in the urine of pernicious anemia patients when given simultaneously with the oral Co⁶⁰ vitamin B₁₂.

This test can be done repeatedly on pernicious anemia patients in complete remission. The limiting factor is the safe dose of Co⁶⁰.

Madison, Wis

111 Callender S T, Turnbull A and Wakasaka G Estimation of intrinsic factor of Castle by use of radioactive vitamin B₁₂ Brit M J 1 10-13 Jan 2 1954

Absorption of vitamin B₁₂ has been assessed by measuring fecal radioactivity after an oral dose of 0.5 mcg of Co⁶⁰ vitamin B₁₂. In 10 normal subjects a mean of 31.0 per cent (standard deviation 6.9 per cent) of the radioactivity was recovered. In 13 patients with pernicious anemia a mean of 88.7 per cent (standard deviation 6.8 per cent) was recovered. When intrinsic factor was given with the radioactive vitamin B₁₂ to 8 patients with pernicious anemia the percentage recovered was reduced greatly.

Radcliffe Infirmary
Oxford England

- 112 Yamamoto, R S and Chow, B F
A rapid method for the determination of B_{12} binding power in the gastric juice *J Lab & Clin Med* 43 316-320, Feb 1954

Authors summary and conclusion

Vitamin B_{12} combines with one or more substances in gastric juice in such a manner as to make it unadsorbable by resting cells of *L. leichmannii*. This fact enables the development of a method for the measurement of the binding power of the gastric juice. With this procedure it was demonstrated that specimens obtained from pernicious anemia patients and from old individuals without achlorhydria had significantly lower binding power than control specimens. Gastric juice specimens from three cases of juvenile pernicious anemia and from old people with achlorhydria contained at least as much [binding power] as the normal samples. The importance of such findings is discussed.

*School of Hygiene and Public Health
Johns Hopkins University
Baltimore Md*

- 113 Glass G B J Uptake of radioactive vitamin B_{12} by the liver: test for intestinal absorption of vitamin B_{12} and measure of the intrinsic factor activity *Clin Research Proc* 2 32, March 1954

By scintillation measurement of the hepatic uptake of ingested Co^{60} vitamin B_{12} in 8 controls and 8 patients with macrocytic anemias a test procedure was developed for quantitation of intestinal utilization of vitamin B_{12} in humans and measurement of intrinsic factor activity. In nutritional and hemolytic anemias with normal gastric secretion hepatic uptake of ingested radioactive vitamin B_{12} was normal, but in 3 patients with pernicious anemia in relapse and remission it was negligible or nil. This indicated defective transport of ingested vitamin B_{12} to the liver in pernicious anemia. The liver uptake became normal after the same dose of Co^{60} vitamin B_{12} was taken with normal human gastric juice. Thus the ability

of normal gastric juice to promote the transfer of vitamin B_{12} through intestinal wall to the liver was directly demonstrated. The reason for intestinal block to utilization of vitamin B_{12} in pernicious anemia patients is the absence of intrinsic factor from their gastric juice.

In sprue hepatic uptake of vitamin B_{12} was also negligible, but it was not corrected by addition of normal human gastric juice or intrinsic factor concentrate. This indicated that the absorption defect in sprue was not caused by lack of intrinsic factor in the gastric juice but by a defect in the intestinal wall itself.

*New York Medical College
New York N Y*

- 114 Schilling, R F Recent studies of intrinsic factor and the utilization of radioactive vitamin B_{12} , *Federation Proc* 13 769-775, Sept 1954

The author reviews the methods employing radioactive vitamin B_{12} that have been used to corroborate the fact that intrinsic factor enhances the utilization of orally administered vitamin B_{12} . After discussing the chemical nature of the intrinsic factor the conclusion is that although intrinsic factor is a mucoprotein, not all mucoprotein in gastric juice is intrinsic factor.

In summary it is stated that intrinsic factor probably exerts a unique type of biological activity for no other substances are known which are secreted exclusively to enhance absorption of a specific vitamin. It is suggested however that there may be intrinsic factors for other essential nutrients.

*University of Wisconsin Medical School
Madison Wis*

- 115 Baker S J and Mollin D L The relationship between intrinsic factor and the intestinal absorption of vitamin B_{12} , *Brit J Haemat* 1 46-51, 1955 (abstr *Am J Clin Path.* 25 1200 Oct 1955)

Vitamin B_{12} labeled with Co^{60} (half life 72 days) was given to patients with

pernicious anemia in remission and fecal radioactivity was measured. Over a certain range there was a stoichiometric relationship between the amount of vitamin B₁₂ absorbed and the amount of intrinsic factor administered. Beyond this range markedly increased amounts of intrinsic factor produced no increased absorption of the vitamin. Individuals varied in ability to absorb vitamin B₁₂ administered with a given amount of intrinsic factor. Such variations suggest that the development of vitamin B₁₂ deficiency depends not only on failure of intrinsic factor production but also on inability to absorb vitamin B₁₂ intrinsic factor complex.

- 116 Glass G H J Boyd L J Stephenson L and Jones E L. Metabolic interrelations between intrinsic factor and vitamin B₁₂. III B₁₂ absorption at varied intrinsic factor doses. *Proc Soc. Exper Biol & Med* 88 15 Jan. 1955

The authors administered varying dosages of intrinsic factor with constant dosage of Co⁶⁰ vitamin B₁₂ to 8 patients with pernicious anemia in remission to 1 patient with total gastrectomy and to 1 control.

Up to a certain point increasing the oral dosage of intrinsic factor while the dosage of vitamin B₁₂ remained constant increased the intestinal absorption of the vitamin, as measured by the hepatic uptake of Co⁶⁰ vitamin B₁₂. Further increase in the dosage of intrinsic factor however may decrease intestinal absorption of vitamin B₁₂. Unless the dosage ratio of vitamin B₁₂ to intrinsic factor is optimal results of assays to determine intrinsic factor potency may be erroneous.

New York Medical College
Flower and Fifth Avenue Hospitals
New York N Y

- 117 Rabiner S F Ellenbogen L, Lichtman H C Williams W Kabakow H and Watson R J. Urinary excretion of vitamin B₁₂ Co⁶⁰ as a method

of assay for intrinsic factor. *Clin Research Proc* 3 28 29 Feb 1955

Patients with pernicious anemia in remission were given orally 2 mcg of Co⁶⁰ vitamin B₁₂ and simultaneously injected intramuscularly with 1.0 to 4.0 mg of unlabeled vitamin B₁₂. Urine collected for the next 24 hours was measured for radioactivity. Subsequently animal intrinsic factor was fed to the patient along with the radioactive vitamin B₁₂ and the injected nonradioactive vitamin B₁₂. Little or no radioactivity was found in the first 24 hours in the urine of patients with pernicious anemia. Under the same conditions normal persons excreted 5 to 20 per cent (average 11 per cent) of the radioactive dose administered. When patients with pernicious anemia were given the clinically active source of intrinsic factor however their average urinary excretion values were approximately normal. Intrinsic factor preparations of submaximal clinical activity produced urinary excretion values in such patients between 1 and 5 per cent. The data indicate that the assay of intrinsic factor can be performed rapidly and accurately using patients with pernicious anemia in remission.

St. Louis University of New York College of Medicine
New York N Y
Lederle Laboratories
Penikese Island N Y

- 118 Ellenbogen L, Williams W L Rabiner S F and Lichtman H C. An improved urinary excretion test as an assay for intrinsic factor. *Proc Soc Exper Biol & Med* 89 357 362 July 1955

A procedure is suggested for assaying intrinsic factor preparations by measuring urinary radioactivity following oral dosage with Co⁶⁰ vitamin B₁₂. More than one intramuscular flushing dose of unlabeled vitamin B₁₂ is given to produce maximum excretion of radioactivity. With this method the same patient can be used for assaying several preparations of intrinsic factor in succession. The injection

of unlabeled vitamin B₁₂ in doses several times as large as the usual flushing dose appeared to decrease the uptake of subsequent small oral doses of labeled vitamin B₁₂ for reasons not fully understood. There appeared to be some correlation between the dosage of intrinsic factor and the amount of radioactivity excreted. Patients with pernicious anemia and normal persons were tested.

*American Cyanamid Co. and
Lederle Laboratories
Pearl River, N. Y.*

*State University of New York College of Medicine
New York, N. Y.*

- 119 Bishop, R. C., Toporek, M., Nelson, N. A., and Bethell, F. H. The relationship of binding power to intrinsic factor activity, *J. Lab. & Clin. Med.* 46:796, Nov. 1955 (in Soc. Proc.)

"The role of intrinsic factor in the absorption of vitamin B₁₂ has long been under investigation. Some substances with intrinsic factor activity bind B₁₂ and make it unavailable for growth in the microbiologic assay for the vitamin. The relationship of this binding power to intrinsic factor activity has not yet been definitely established. When pernicious anemia (PA) patients were used to evaluate the intrinsic factor activity of experimental preparations there was an incomplete correlation between such activity and the binding power of these preparations. One of the difficulties presented by the use of PA patients is the fact that the gastric juice of such patients does have some binding power.

To circumvent the possible effect of the binding power of the PA patients' own gastric juice, the binding power in intrinsic factor relationship was studied in patients with total gastrectomies using the urinary excretion of orally administered radioactive B₁₂-Co⁶⁰ as a measure of absorption. A control test: oral B₁₂-Co⁶⁰ without intrinsic factor was followed by other tests at weekly intervals. In one oral dose of B₁₂-Co⁶⁰ was followed immediately

by an oral dose of normal human gastric juice (GJ) which had been incubated for 30 minutes at room temperature with equivalent amount of nonradioactive B₁₂. In the second part of the experiment, the B₁₂-Co⁶⁰ was bound to GJ and the nonradioactive B₁₂ was free. As a check on the effect of prolonged contact apart from binding on absorption a test was run with B₁₂-Co⁶⁰ and gastric juice administered separately without prior incubation. The findings confirmed earlier observations that prior incubation does not enhance absorption.

The data indicate that the B₁₂ already bound by the GJ is preferentially absorbed. One series of tests on a PA patient gave similar results. It is concluded that binding power is necessary for intrinsic factor activity.

Ann Arbor, Mich.

- 120 Bull, F. E., Campbell, D. C., and Owen, C. A., Jr. Evaluation of intrinsic factor in pernicious anemia by means of cobalt 60-vitamin B₁₂. *Federation Proc.* 15:509-510, March 1956.

Absorption of orally administered Co⁶⁰ vitamin B₁₂ may be estimated by measurement of (1) radioactivity in feces, (2) radioactivity in the liver, or (3) radioactivity in urine after a flushing dose of parenterally administered unlabeled vitamin B₁₂. These methods were used to study intrinsic factor activity in 92 patients. A sharp distinction in absorption of vitamin B₁₂ was evident by all techniques when normals were compared to patients with pernicious anemia. The urinary method was simplest and quickest but unrecognized loss of part of the specimen might suggest pernicious anemia in a normal person. In patients with uremia renal excretion of Co⁶⁰ often was so retarded that a single 24-hour urinalysis gave misleadingly low results; however, total excretion over a period of several days was normal. The fecal-excretion method was accurate but laborious; here unrecognized loss of specimens might

yield false normal values. Hepatic radioactivity was measurable only after unabsorbed intestinal Co^{57} was excreted; this method was slow but perhaps most accurate since it did not depend on the patient's cooperation in collecting excreta. When initial tests indicated lack of intrinsic factor they were repeated with the addition of gastric juice to the labeled vitamin patients with pernicious anemia then showed normal absorption but patients with severe sprue or intestinal disease often did not.

Mayo Foundation and
Mayo Clinic
Rochester, Minn

- 21 Best W R White W F Robbins A C Landmann W A and Steelman S L. Studies on urinary excretion of vitamin B_{12} Co^{57} in pernicious anemia for determining effective dosage of intrinsic factor concentrates. *Blood* 11 338-351 April 1956

In order to assay intrinsic factor concentrates and to determine optimal dosage schedules for oral therapy urinary excretion of Co^{57} vitamin B_{12} was determined in patients with pernicious anemia. Co^{57} vitamin B_{12} was given orally with intrinsic factor and followed by a flushing parenteral dose. Assay of intrinsic factor by this method is valid only if the ratio of intrinsic factor to inactive vitamin B_{12} binding material is reasonably constant and if the intrinsic factor contains insignificant amounts of vitamin B_{12} .

The authors found that increasing doses of intrinsic factor concentrates give increasing excretions of radioactivity with low doses of vitamin B_{12} . Little additional increase with moderate doses and sometimes a diminution with excessive doses. Assay by urinary excretion was compared with assay by hematologic response in 13 patients with pernicious anemia in relapse. The two tests showed some correlation but individual values varied greatly. The authors state that lack of a pure intrinsic factor and variations in individual patients make it impossible to formulate

the exact relationship of intrinsic factor dose vitamin B_{12} dose and intestinal absorption of vitamin B_{12} .

University of Illinois College of Medicine
Chicago, Ill

- 122 Johnson P C Richmond V, Caputto R and Wolf S. Fractionation of human gastric content: the separation of intrinsic factor activity. *J Clin Investigation* 35 716 June 1956 (in Soc Proc)

A method of identification of intrinsic factor in material from the fractionation of human gastric contents (chromatography) was studied in 4 patients with peptic ulcer and in 4 with pernicious anemia and in healthy subjects. In patients with peptic ulcer and in normal subjects seven liters of effluent yielded 50 to 75 per cent recovery. The first liter of effluent contained the major portion of the protein and carbohydrate in two well defined peaks. Only the fourth liter contained intrinsic factor activity however and here the zone of activity was narrowed down to a single 400 cc fraction. From this fraction as little as 10 mg of dried material produced absorption of the tagged vitamin B_{12} in subjects in whom no absorption had been demonstrated in control runs. These experiments provide the first step toward the establishment of a normal fractional pattern of human gastric content. Such a pattern would not only allow for the direct recognition of missing biologically active components but like the fractions of blood serum might ultimately be useful in differential diagnosis.

Oklahoma City, Okla

- 123 Chow B F Quattlebaum J K Jr and Rosenblum C. Effect of intrinsic factor concentrate on vitamin B_{12} absorption by gastrectomized rats. *Proc Soc Exper Biol & Med* 90 279-281, Oct 1955

Radioactive vitamin B_{12} with and without exogenous intrinsic factor was

administered orally to totally gastrectomized rats. Absorption of vitamin B₁₂ was measured by scintillation counting of the feces. No absorption occurred in the gastrectomized rats whether or not intrinsic factor was given. Intrinsic factor inhibited the absorption of vitamin B₁₂ by control rats.

The authors suggest that rats can utilize only endogenous intrinsic factor and that exogenous intrinsic factor is ineffective or inhibitory. The studies may also indicate that the stomach is the major site of vitamin B₁₂ absorption by the rat. Studies of the same type will be made using rat stomach concentrate. If this concentrate proves effective it will demonstrate species specificity of intrinsic factor.

On a weight basis the rat appears capable of absorbing greater quantities of vitamin B₁₂ than the human. Thus in the absence of intrinsic factor absorption from an oral dose of 50 micromicrograms amounts to 20 to 25 micromicrograms and from 200 micromicrograms absorption is 30 micromicrograms. The equivalent oral doses for a 60 Kg human would be 10 mcg and 40 mcg and the corresponding absorption 4 to 6 mcg. Actually the human absorbs only 1.5 mcg from such doses.

*Johns Hopkins University
Baltimore Md*

*Merck & Co
Rahway N J*

124 Nieweg, H O, Arends, A, Mandema, E and Castle W B. Enhanced absorption of vitamin B₁₂ in gastrectomized rat by rat intrinsic factor, *Proc Soc Exper Biol & Med* 91: 328-332 Feb 1956.

In preliminary studies to develop an animal preparation for the detection of intrinsic factor, male rats were totally gastrectomized and anastomosis of the esophagus to the pylorus was done. Rats in good health 10 days after the operation were fed a low residue synthetic diet containing all essential nutrients including vitamin B₁₂. Radioactive vitamin B₁₂ 0.015 mcg in 1 cc of water, and intrinsic factor preparations were given by stomach tube. Feces assayed for vitamin B₁₂ showed that normal rats absorbed a significant amount while gastrectomized rats absorbed none. Both normal human gastric juice in doses of 2 to 6 cc, and hog stomach mucosa in doses of 4 to 20 mg were ineffective in improving vitamin B₁₂ absorption. The authors conclude that assimilation of vitamin B₁₂ in the rat in some ways parallels that in man and that the negative results obtained with intrinsic factor preparations from other species may point to species specificity for intrinsic factor preparations.

*Boston City Hospital
Harvard Medical School
Boston, Mass*

*University of Groningen
Groningen, Holland*

LIVER

VITAMIN B₁₂ AND LIVER DISEASE

125 Jones P N and Mills E H. Serum vitamin B₁₂ concentrations in liver disease. *J Lab & Clin Med* 46: 927, Dec 1955 (in Soc Proc.)

Serum vitamin B₁₂ concentrations were studied in 11 patients with Laennec's cir-

rhosis in 10 with postnecrotic cirrhosis in 4 with biliary cirrhosis, in 1 with acute viral hepatitis and in 4 in hepatic coma. Those with biliary cirrhosis had values within the normal range all others had vitamin B₁₂ serum concentrations at least 3 to 8 times normal. Patients in hepatic coma had values between 30 and 40 times normal. Patients with Laennec's cirrhosis had proportionate rises in both the free

combined vitamin B₁₂ while those with acute hepatitis or postnecrotic cirrhosis had a greater proportional rise in serum vitamin B₁₂. Determinations made on 24 hour collections showed that these patients excreted from 10 to 30 times the normal amount of vitamin B₁₂ all in the urine.

It is concluded that the metabolism of vitamin B₁₂ is seriously altered in both acute and chronic liver disease.

126

Stevenson T D and Beard M F. Hepatic uptake of radioactive vitamin B₁₂ in liver disease. Federation Proc. 15 74-March 1956

To evaluate uptake of vitamin B₁₂ by diseased liver 35 measurements with vitamin B₁₂ were performed in 15 patients with diffuse liver disease. The hepatic uptake was measured by scintillation counting the surface of the liver following parenteral or oral administration of Co⁶⁰ vitamin B₁₂ alone or with potent intrinsic factor preparation. The data obtained at various stages of disease correlated with liver function tests and the clinical picture. The hepatic uptake of orally administered Co⁶⁰ vitamin B₁₂ was temporarily abolished in 1 patient with severe virus hepatitis as well as in 1 patient with severe cirrhosis and in 1 with obstructive jaundice. Of 3 others with hepatitis, 1 with cirrhosis and 3 with obstructive jaundice the hepatic uptake of Co⁶⁰ vitamin B₁₂ was at a low level in 6. In 4 out of 5 cases of metastatic or primary liver carcinoma however the hepatic uptake was normal both on oral and parenteral administration.

The addition of intrinsic factor preparation in 8 out of 9 tests did not improve decreased hepatic uptake of Co⁶⁰ vitamin B₁₂. Thus decreased uptake obviously does not depend on the decreased output of intrinsic factor but on the im-

paired ability of the diseased liver to pick up or retain vitamin B₁₂. It appears however, that an advanced lesion does not always severely impair the liver's ability to anchor vitamin B₁₂ especially after parenteral administration.

New York Medical College
New York N Y

- 127 Stevenson T D and Beard M F. Observations on the metabolism of vitamin B₁₂ in patients with liver disease. Clin Research Proc 4 144, April 1956

Studies on patients with liver disease showed that levels of serum vitamin B₁₂ were elevated only in those with hepatic parenchymal disease i.e. alcoholic cirrhosis and infectious hepatitis and that the serum level of vitamin B₁₂ was markedly elevated in patients in hepatic coma. Patients with obstructive jaundice did not show increased serum levels of vitamin B₁₂.

Observations indicate that return of the serum vitamin B₁₂ level to normal parallels clinical improvement. The evidence suggests that the elevation of the serum level of vitamin B₁₂ in patients with liver disease represents the release of the vitamin from hepatic cells.

University of Louisville
School of Medicine
Louisville Ky

- 128 Rachmilewitz, M, Aronovitch J and Grossowicz N. Serum concentrations of vitamin B₁₂ in acute and chronic liver disease. J Lab & Clin Med 48 339 344 Sept 1956

High vitamin B₁₂ values were found in the sera of 13 of 14 patients with acute viral hepatitis. Concentrations ranged from 800 to 1000 micromicrograms per cc and decreased gradually with subsidence of jaundice. In 5 cases of biliary cirrhosis and in 3 of 6 cases of portal cirrhosis the vitamin B₁₂ concentration ranged from 830 to 3600 micromicrograms per cc. In 6 cases of extrahepatic biliary obstruction and jaundice however

the serum vitamin B₁₂ was within normal range

The maximal binding capacity (MBC) of the serum of normal individuals ranged from 1 200 to 3 000 micromicrograms per cc. In patients with liver disease and high serum vitamin B₁₂ it ranged from 1,000 to 3 800 micromicrograms per cc.

The increase of serum vitamin B₁₂ in liver disease is considered by the authors to be due to the release of the stored vitamin from damaged liver cells.

Hebrew University
Hadassah Medical School
Jerusalem Israel

- 129 Baker H, Pasher I, Dolger H P and Sobotka, H Vitamin B₁₂ excretion as index of hepatic disorder, Clin Chem 2 328 330 Oct 1956

This paper reports the decreased renal excretion of intramuscular vitamin B₁₂ in patients with liver involvement, and the gradual increase to normal levels during the period of convalescence. Urinary excretion of vitamin B₁₂ using *L. leichmannii*, *Euglena gracilis* and *Ochromonas malhamensis* assays was determined for 20 patients with liver disease and for 3 controls following a single intramuscular load dose of 50 mcg of vitamin B₁₂.

Normal controls excreted 24 to 40 mcg of the 50 mcg dose while patients with liver disease excreted 10 mcg or less. Some patients considered convalescent also failed to excrete normal amounts of vitamin B₁₂. Four patients recovering from pneumonia also excreted less than 10 mcg of vitamin B₁₂ indicating the effect of the infection on the liver.

It is stated that a high excretion of vitamin B₁₂ after a load dose would indicate that the liver was again capable of storing the vitamin and that increased amounts from injection are no longer required for normal metabolic activity of the organ.

The various liver disorders are being categorized by determining the extent to which vitamin B₁₂ excretion is correlated with liver damage. This may aid in de-

tecting how extensive the liver damage is and how far convalescence has progressed.

Mount Sinai Hospital
New York N Y

- 130 Ostergaard Kristensen H P The vitamin B₁₂ concentration in plasma from patients with acute hepatitis and obstructive jaundice, Nord med 55 85 87, 1956 (abstr Blood 11 1055 1056 Nov 1956)

The plasma concentration of vitamin B₁₂ was determined microbiologically in patients with acute hepatitis, obstructive jaundice and hepatic cirrhosis. *Lactobacillus leichmannii* 313 was used as the test organism. In the active phase of acute hepatitis a remarkable increase in plasma vitamin B₁₂ activity was found. In obstructive jaundice and hepatic cirrhosis normal or slightly increased values were found.

University of Copenhagen
Biochemical Institute
Copenhagen Denmark

- 131 Posteraro P The treatment of minor hepatic dysfunction in children, Minervapediat 6 32 Jan 15 1954 (abstr A M A Am J Dis Child 91 399, April 1956)

Signs of minor hepatic dysfunction in children may often follow infection where sulfonamides or antibiotics have been given. The symptoms may include pallor, subicteric sclerae, slightly enlarged liver, asthenia, anorexia, fat indigestion and urticaria. The author has treated 50 children with this condition with very favorable results by a preparation containing betaine, choline and vitamins B₁ and B₁₂.

- 132 Canivell F Viral hepatitis: some pediatric aspects Arch pediat Barcelona 4 379 387 Jan-Feb 1954 (abstr A M A Am J Dis Child 89 753 June 1955)

The writer discusses the epidemiology, symptomatology, diagnosis and differen-

tial diagnosis of viral hepatitis. Importance of rest is emphasized. Chlorotetracycline and oxytetracycline and choline and methionine are described as effective but must not be given in too large doses. Vitamin B₁₂, folic acid, yeast and a diet high in protein and carbohydrates but exceedingly poor in fats should be maintained as long as needed.

- 133 Colwell A R Jr Occurrence of accumulation of fat in the liver and its relation to excess weight gain in patients convalescing from viral hepatitis. *Ann Int Med* 41 963 979 Nov 1954

In 144 soldiers convalescing from viral hepatitis liver biopsy showed fat accumulation in the liver of about half. Those showing liver fat gained more than twice as much body weight during convalescence as those showing no abnormal fat in the liver. The amount of liver fat in these hepatitis patients did not approach that seen in alcoholic cirrhosis. Fat deposition never occurred in the acute phase of hepatitis but was seen 6 to 13 weeks after onset of the disease.

About 60 patients had been given vitamin B₁₂ 30 mcg every other day (apparently intramuscularly) during the very early phases of hepatitis. 8 received 15 mcg every other day for four weeks. This failed to prevent abnormal accumulation of liver fat and did not shorten convalescence. The author says however that use of vitamin B₁₂ might be justified to increase appetite.

The author believes that increased intake of calories and fat and perhaps a relative protein deficiency were responsible for the fat deposition. Hepatic function as estimated by the bromsulphalein retention test was not impaired by the accumulation of fat and the incidence of reticulosis was not increased. Although it is impossible to state whether this fat is harmful its presence on histologic section is abnormal. Therefore the

dietary fat and caloric intake should be moderately limited so that obesity does not occur.

*U S Army Hospital
Kyoto Japan*

- 134 Campbell R E and Pruitt F W The effect of vitamin B₁₂ and folic acid in the treatment of viral hepatitis. *Am J M Sc* 229 8 15 Jan 1955

A control group of 44 patients with acute viral hepatitis received conventional treatment consisting of high protein high carbohydrate moderate fat diet and bed rest. Another group of 44 patients comparable in race age duration of illness and degree of icterus received the same treatment plus 30 mcg of vitamin B₁₂ by intramuscular injection every other day and 5 mg of folic acid by mouth three times a day for the first 10 days of hospitalization.

Patients who received vitamin B₁₂ and folic acid regained normal appetite more rapidly than those given routine treatment. Average duration of illness in the experimental group was 47.5 days compared to 57.2 days for the control group.

*U S Army Hospital
Kyoto Japan*

ANIMAL STUDIES

- 135 Shils M E and Stewart W B Development of portal fatty liver in rats on corn diets: response to lipotropic agents. *Proc Soc Exper Biol & Med* 85 298 303 Feb 1954

Authors summary: Rats subsisting on a diet containing 76% corn and 3% or less casein developed a fatty liver characterized by initial and preponderant accumulation of lipid in the portal areas. Rats of the same strain on a diet in which the only protein was casein developed a centrilobular type of fatty liver. Methionine, choline and vitamin B₁₂ were able to decrease the liver lipid. However methio-

nine was rarely capable of preventing the deposition of some excess fat while under certain conditions the lipotropic action of vitamin B₁₂ was unpredictable

*Columbia University College of Physicians and Surgeons
New York N Y*

- 136 Bennett, M A, Hellerman, J and Donnelly, A J Liver lesions due to prolonged feeding of a 'labile methyl' free diet and the protective influence of vitamin B₁₂ Proc Am A Cancer Research 1 4-5, April 1954

The authors anticipated that prolonged feeding of rats on a labile methyl and vitamin B₁₂ free diet might produce neoplastic changes in the liver similar to those in rats fed a choline-deficient diet (*Am J Path* 22 1059 1946)

Twenty four female Lankenau Wistar rats were fed a cystine-free diet of 30 per cent corn oil and 17 per cent amino acids with (group 1) or without (group 2) vitamin B₁₂. Other B vitamins were fed daily. Group 1 maintained normal liver lipids, an average of 0.15 mcg vitamin B₁₂ per Gm dry liver and high body fat. Group 2 showed high liver lipids, low vitamin B₁₂ (average 0.03 mcg per Gm dry liver) and low body fat.

Without vitamin B₁₂ some damage occasionally severe, was present as early as one year after start of experiment with vitamin B₁₂. Morphologic evidence of liver disease appeared only in rats on the diet for over 2 years and these lesions were minimal. Livers of rats on the vitamin B₁₂ free diet showed focal necrosis, fatty metamorphosis, cirrhosis, duct hyperplasia or cysts. Distortions of hepatic architecture in several animals simulated changes which often accompany neoplasms but no lesion could be unequivocally classified as a tumor.

*Lankenau Hospital Research Institute and
Institute for Cancer Research
Philadelphia Pa*

- 137 Schweigert, H S, Scheid H E and Downing M Liver changes in vita

min B₁₂ and riboflavin-deficient rats before and after partial hepatectomy, *Am J Physiol* 178 338 340, Aug 1954

To determine the role of vitamin B₁₂ in the synthesis of nucleic acid, rats deficient in vitamin B₁₂ and those given supplemental vitamin B₁₂ were subjected to partial hepatectomy. Comparable studies with rats deficient in riboflavin and those receiving supplementary riboflavin were also conducted.

The studies showed that the desoxy ribonucleic acid and ribonucleic acid per gram of liver weight or liver nitrogen were reduced in vitamin B₁₂ deficiency but not in riboflavin deficiency. Moreover a higher mortality and more limited regeneration of liver tissue occurred in the vitamin B₁₂-deficient rats than in the vitamin B₁₂ supplemented riboflavin deficient or control rats.

These investigations give further evidence of the role of vitamin B₁₂ in nucleic acid synthesis. However the results should be interpreted with caution since different basal rations were used to produce the two vitamin deficiencies.

*University of Chicago
Chicago Ill*

- 138 Rigdon, R H, Couch J R, Brahear, D and Qureshi, R T Effect of vitamin B₁₂ on selenium poisoning in the rat. *A M A Arch Path* 59 66-72 Jan 1955

Authors summary and conclusions: 'Rats given a small amount of selenium in their ration for short intervals 33 and 48 days have a larger amount of vitamin B₁₂ in their livers than have the controls fed the same ration [without selenium].'

'The daily subcutaneous injection of 200 mcg of vitamin B₁₂ in adult rats apparently does not influence the hepatic necrosis that results from the ingestion of a small amount of sodium selenite over a period of 17 weeks. Vitamin B₁₂ does influence however the process of hepatic repair following injury resulting from se

lenium The vitamin B₁₂ content of the liver is decreased in rats with severe hepatic necrosis resulting from selenium intoxication The vitamin B₁₂ content of the liver of rats undergoing regeneration following selenium poisoning is low It is suggested that regenerating hepatic cells either utilize more vitamin B₁₂ than do normal hepatic cells or that they fail to store vitamin B₁₂ in the same manner in which normal hepatic cells do

University of Texas
School of Medicine
Galveston Texas

- 139 Malloy S: Effect of chronic ethanol intoxication on liver lipid content of rats Proc. Soc. Exper Biol & Med 88 246-249 Feb 1955

Vitamin B₁₂ and choline were given to alcohol intoxicated rats to determine the effect of lipotropic agents on such animals

The intoxicated rats showed increased liver fat even when diet was adequate but large doses of vitamin B₁₂ or choline protected them against fatty livers Doses of 2.9 mcg of vitamin B₁₂ daily were ineffective but 6.8 mcg daily was effective

It is stated that these results suggest that alcohol per se has an effect on the liver Since long standing fatty liver may lead to fibrosis and cirrhosis the effect of alcohol may contribute to the development of liver lesions in alcoholics

State University of New York, Upstate Medical Center
Syracuse N Y

STRESS AND TOXIC STATES

- 140 Hayes M A Water soluble vitamin requirements in surgical convalescence Ann. Surg 140 661-667, Nov 1954

The author emphasizes the importance of adequate nutrition in relation to surgical procedures and to convalescence after operations

Many patients undergoing major surgical procedures particularly gastrointestinal operations have been nutritionally depleted for a considerable period Inadequate feeding following operation aggravates the effect of loss of vital nutrients

Case histories include records of metabolic studies both before and after intensive vitamin therapy (ascorbic acid thiamine riboflavin, niacinamide pyridoxine calcium pantothenate vitamin B₁₂, folic acid and vitamin K) As a result of such therapy carbohydrate metabolism is turned to normal hepatic function and hemoglobin synthesis improved and nitrogen balance became positive

Yale University
School of Medicine
New Haven Conn

- 141 Sebrell W H Jr Adequate therapeutic diets J Am Dietet A 30 1256-1259 Dec 1954 (in Current Comment)

Adequate therapeutic diets are discussed with relation to the patient's need for calories protein vitamins and minerals

In the treatment of illness and trauma the vitamins play an important part Thiamine and niacin are useful in carbohydrate metabolism while riboflavin pantothenic acid pyridoxine folic acid and vitamin B₁₂ participate in protein and energy metabolism as well as in other processes Ascorbic acid also plays a prominent part in therapeutic nutrition

Vitamin B₁₂ is beneficial in very large doses in cranial nerve neuralgias and in diabetic neuritis Spectacular results with vitamin B₁₂ therapy in neurologic disorders of alcoholism have recently been described In animal studies preliminary findings indicate that vitamin B₁₂ may also promote wound healing

National Institutes of Health
Bethesda Md

- 142 Ershoff, H H Decreased resistance of vitamin B₁₂ deficient rats to cold stress, *Proc Soc Exper Biol & Med* 84 615-617, Dec 1953

Author's summary '1 Prolonged exposure to cold significantly increased the vitamin B₁₂ requirement of the rat 2 Resistance to cold stress as measured by length of survival under conditions of low environmental temperature was significantly impaired in the vitamin B₁₂-deficient rat

Emory W Thurston Laboratories
Los Angeles Calif

- 143 Kleinsorge, H, Morigerowski, E and Rosner K Anemia in lead poisoning and vitamin B₁₂, *Ztschr ges inn Med* 9 903, Sept 15 1954 (abstr *A M A Arch Indust Health* 12 452 453, Oct 1955)

In rabbits rendered anemic by injections of aqueous lead acetate solution administration of vitamin B₁₂ with or immediately after the lead dosage resulted in a much more rapid recovery from the anemia Stimulation of the regenerative power of the bone marrow was the cause of the more rapid recovery Vitamin B₁₂ also prevented deterioration of the general condition of the rabbits When lead was given with vitamin B₁₂ basophilic stippling of the erythrocytes was prevented The authors feel that the vitamin B₁₂ either stimulates normal maturation preventing liberation of immature stippled cells or that because it is important in nucleic acid metabolism, it normalizes a pathologic process of ripening which may result from inhibition of the nucleic acid metabolism by lead

- 144 Hara M Experimental study on the treatment of wounds in cases of hypoproteinosis Nagoya *J M Sc* 16 12 1953 (*Internat Abstr Surg* 99 520 1954, in *Surg Gynec & Obst*, Nov 1954)

The rate and character of healing of simple skin scratches linear incisions and circular skin defects from 5 to 10 mm

in diameter were observed in a total of 262 normal and hypoproteinemic mice Hypoproteinemic mice showed poor healing The wound reaction was localized and limited and fibroblasts appeared as late as five days after wounding normal mice showed good fibroplasia by the second and third days Regeneration of blood vessels was also delayed in the wounds of hypoproteinemic mice The only amino acids which had beneficial effects when given singly were methionine, lysine and tryptophan, in the order listed When amino acids with vitamin B₁₂ and folic acid were added to the diets of hypoproteinemic mice wound healing approached normal

- 145 Mecray, P Jr Nutrition and wound healing *Am J Clin Nutrition* 3 461 465, Nov Dec 1955

The effect of protein and vitamin deficiencies on wound healing is reviewed The fat soluble vitamins presumably play a role in the healing of bone wounds, and the B-complex vitamins are essential to wound healing acting as coenzymes Deficiency of pyridoxine and riboflavin delayed wound healing in rats Vitamin B₁₂ increased the strength of rat wounds in the early stages of healing

Cooper Hospital
Camden N J

- 146 Bloodworth, J M B Jr and Hamwi G J The effect of diet antibiotics and vitamin B₁₂ on experimental glomerular lesions produced by cortisone *J Clin Endocrinol & Metab* 16 944 July 1956 (in *Soc Proc*)

Previous research showed that administration of cortisone acetate to rabbits caused glomerular lesions resembling those in human beings with diabetes mellitus The authors continued their research by giving to rabbits feeds containing crude antibiotic vitamin B₁₂ supplement The results were fewer lesions and fewer animals showing lesions Other drug regimens did not produce this effect

Ohio State University College of Medicine
Columbus Ohio

- 147 Bereston E S Vitamins in dermatology *Am J Clin Nutrition* 2 133 139 March April 1954

The author discusses the use of vitamins in cutaneous diseases. Vitamin B₁₂ in his experience relieves the pain of herpes zoster; good results are also reported in the treatment of seborrheic dermatitis and chronic discoid lupus erythematosus. Transient but marked improvement occurs in subacute disseminated lupus erythematosus.

*University of Maryland School of Medicine
Baltimore Md*

- 148 Musman B J Psoriasis seborrheic dermatitis and pityriasis rosea *Post grad Med* 17 85 92 Jan 1955

The causes and treatment of psoriasis seborrheic dermatitis and pityriasis rosea are discussed.

After reviewing the results of local application of ointments and of systemic therapy including cortisone and corticotropin, the author suggests vitamin B complex, liver, riboflavin, and vitamin B₁₂ as valuable adjuncts in the treatment of these skin diseases.

*University of Colorado School of Medicine
Denver Colo*

- 149 Barefoot S W A method of treating chronic leg ulcers *North Carolina M J* 16 101 102 March 1955

Twenty-three patients with chronic stasis ulcers of the leg, secondary to varicose veins or to old thrombophlebitis, were treated locally with antibiotic powder and absorbable gelatin and given vitamin B₁₂ parenterally 30 to 45 mcg once a week. All ulcers healed satisfactorily within 60 to 75 per cent of the time required when absorbable gelatin and vitamin B₁₂ were not used.

An ulcer of the nose, resulting from severe radiodermatitis, healed within four months on the regimen of antibiotics, gelatin and vitamin B₁₂. Conventional ther-

apy for two years had failed to effect a cure.

Greensboro N C

- 150 Goldblatt S On the intravenous administration of cyanocobalamin *Am J Clin Nutrition* 3 129 131, March April 1955

No adverse reactions were noted in the course of 3 297 intravenous injections of vitamin B₁₂ in 150 patients with various skin, neurologic or endocrine diseases. The dosage ranged from 15 to 3 000 mcg.

The patients' ages ranged from 4 to 78 years. Daily injections of 1 000 mcg were given to a 10-year-old girl for over 270 days without toxic reactions. The largest single dosage used (3 000 mcg) was given daily to a patient for a total of 86 days. One patient described burning of the tongue, headache and postnasal drip 10 minutes after one injection of 1 000 mcg, although she had no other reaction to numerous injections given before and afterward.

*University of Cincinnati Medical School
Cincinnati Ohio*

- 151 Neber H Die Therapie verschiedener Hautkrankheiten insbes allergischer Genese mit Vitamin B₁₂ [The treatment of various skin diseases particularly those of allergic etiology with vitamin B₁₂] *München med Wchschr* 97 1102 1103 Aug 26 1955

Vitamin B₁₂ was used in the treatment of 41 patients with diseases of the skin. Ampules containing 1 000 mcg of the vitamin, presumably for intramuscular injection, were used.

Of 11 patients with contact dermatitis, 9 improved enough to be in contact with the offending allergen without reaction. These patients received 6 000 mcg of vitamin B₁₂ in 10 to 14 days.

Dermatitis medicamentosa caused by

penicillin-streptomycin in 5 patients and by topical iodine in 4, healed after vitamin B₁₂ 1,000 mcg, was given for two days followed by 500 mcg for two days. Though such healing could have occurred spontaneously, it is significant that 8 of these patients could continue receiving the offending medication. Of 8 patients with eczema of endogenous etiology who received injections of 1,000 mcg of vitamin B₁₂, 4 were relieved but had recurrences. 4 were free of itching but lesions remained.

Five patients had eczema of the legs associated with varicose ulcers which were being treated with a zinc lime dressing. They could tolerate the dressings without itching and rash when they were given two injections of 1 000 mcg of vitamin B₁₂ and three later injections of 500 mcg. Two patients with severe juvenile acne vulgaris received injections of 1,000 mcg of vitamin B₁₂ every other day for 10 days followed by five more injections of 500 mcg every other day. In both cases the skin cleared and lesions have not returned in six months.

Rosacea cleared in 4 of 5 women who received 500 mcg of vitamin B₁₂ every other day for two to three weeks.

A man with lichen ruber planus was given two injections of 1,000 mcg of vitamin B₁₂ a week for four weeks and then once a week for eight weeks. His lesions cleared. A recurrence six months later responded to further therapy.

The author mentions impressive results obtained by Ruedemann with vitamin B₁₂ 1,000 mcg daily in 34 patients with psoriasis.

Berlin Charlottenburg Germany

- 152 Goldblatt, S. Lupus erythematosus treated with vitamin B₁₂. Acute systemic lupus erythematosus, chronic discoid lupus erythematosus. *A M A Arch. Dermat & Syph* 68 737-739, Dec 1953 (in Soc Proc)

A patient with lupus erythematosus was treated with intramuscular vitamin

B₁₂ 15 to 120 mcg three times weekly throughout pregnancy. She improved with each injection. A recurrence of lupus erythematosus was treated with 120 mcg vitamin B₁₂ twice weekly. Consistent improvement in the general physical condition as well as in the local lesions followed.

Vitamin B₁₂ was well tolerated by other patients in doses ranging from 15 to 1 000 mcg for long periods. The author states that vitamin B₁₂ is a safe, effective agent for the treatment of all types of lupus erythematosus, producing involution of dermal lesions, regression of systemic symptoms, elevation of pili hyposthesia and correction of anemia.

- 153 Marcus, M D, Conrad A H, Jr and Weiss, R S. Treatment of chronic discoid lupus erythematosus with large doses of vitamin B₁₂. *J Invest Dermat* 21 75 77, Aug 1953

Chronic discoid lupus erythematosus in 17 patients was treated with vitamin B₁₂ in intramuscular doses of 1 000 mcg three times a week. In 1 patient there was almost complete clearing of the lesions. 2 patients were greatly improved. 2 slightly improved. 9 were unchanged and 3 became worse. Most of the patients reported a feeling of well being and increased energy.

*Barnard Free Skin and Cancer Hospital
St Louis County Hospital and
Washington University School of Medicine
St Louis Mo*

- 154 Brunsting L A. Systemic lupus erythematosus. *South Dakota J Med & Pharm* 7 311 313 317, Sept 1954

After evaluating corticotropin and cortisone in the treatment of systemic lupus erythematosus the author states that vitamin B₁₂ administered intramuscularly in doses of 15 mg [sic] twice a week for 10 or 12 weeks effects improvement.

*Mayo Clinic
Rochester Minn*

- 155 Robinson R C V Treatment of xanthelasma and vitamin B₁₂ J Invest Dermat 24 111 113 Feb 1955

Vitamin B₁₂ was given subcutaneously to 35 patients and orally in 30 with xanthelasma palpebrarum. The subcutaneous dosage ranged from 30 mcg to 1 000 mcg weekly for 6 to 20 weeks. The oral dose was 1 000 mcg daily for 30 days. A favorable result was obtained in 31 of the 35 patients receiving vitamin B₁₂ subcutaneously but in only 5 receiving it orally.

The process by which vitamin B₁₂ ameliorates xanthelasma is apparently not related to its effect on cholesterol metabolism since cholesterol levels measured before and after subcutaneous or oral vitamin B₁₂ in 65 patients showed no significant change. The reason why oral vitamin B₁₂ was ineffective is not clear since blood level measurements showed that it is absorbed from the gastrointestinal tract.

Baltimore City Hospitals
Baltimore Md

- 156 Nicolini A Treatment of erythroderma desquamativum with vitamin B₁₂ Minerva pediat 8 53 57 Jan 28 1956 (abstr Med Monatsspiegel 5 136 June 1956)

In 14 cases of desquamative erythroderma in infants an average daily dose of 50 mcg of vitamin B₁₂ orally led to striking improvement.

Milan Italy

- 157 Ruedemann R Jr Treatment of psoriasis with large doses of vitamin B₁₂ 1,100 micrograms per cubic centimeter A M A Arch Dermat & Syph 69 738 739 June 1954 (in Clinical Notes New Instruments and Techniques)

The author reports on 34 patients with psoriasis who had been treated with intramuscular vitamin B₁₂ 1 000 mcg daily

for 10 to 20 days followed by a maintenance dose. The eruption subsided in 11 patients. In 10 it was 75 to 80 per cent improved. 5 patients are improving slowly. 2 uncooperative patients were not benefited. 5 patients had slight recurrences but treatment was resumed with good results. Pruritus was relieved in 9 of these patients.

The author states that this treatment has surpassed all previous methods in rapid effectiveness, lack of reactions, and simplicity. The rapid response of patients with long standing recalcitrant psoriasis is too consistent to attribute to spontaneous involution. Wholesale clinical trials with vitamin B₁₂ therefore are indicated.

Albany Medical College
Albany N Y

- 158 Rimbaud P Ravoire J and Rioux J Le traitement du psoriasis par la vitamine B₁₂ a 1 000 gammas [Treatment of psoriasis with vitamin B₁₂ in a dose of 1000 mcg] Presse méd 64 194 Feb 1, 1956 (in Soc Proc)

For treatment of psoriasis injection of 1 000 mcg of vitamin B₁₂ daily for an average of 15 days has been proposed. When the authors used it in 17 cases of resistant psoriasis 9 showed sustained improvement, 3 had some improvement and 5 none. Best results were obtained when there was much congestion and irritation in subsebaceous forms; the response was not as good.

- 159 Stiegler M Traitement du psoriasis par la vitamine B₁₂ [Treatment of psoriasis with vitamin B₁₂] Presse méd 64 1719 Oct 20 1956 (in Soc Proc)

Psoriasis in 30 patients was treated with vitamin B₁₂. The results were as follows: recovery in 3 cases, definite improvement in 10, slight improvement in 8, and complete failure in 9 cases.

Stasbourg France

NUTRITION AND GROWTH IN CHILDREN

- 160 Kofman, I. Acción estimulante general de la vitamina B₁₂ en pediatría [General stimulating effect of vitamin B₁₂ in pediatrics], *Semana méd* 103 261-266, Aug 20 1953

Vitamin B₁₂ was given daily to 23 children, all but 2 of whom were under five years of age. All were underweight and ate poorly, but were free of overt disease. Appropriate changes in diet and administration of calcium salts, liver extracts, vitamins, tonics, and ultraviolet therapy had already been instituted when indicated before vitamin B₁₂ was given.

Daily intramuscular injections of 30 mcg of vitamin B₁₂ were given to 19 children, and 4 received the same daily dosage orally. Total dosage was 300 to 1,850 mcg in 10 to 60 days with an average of 660 mcg in 32 days. Increased appetite was observed in 16 children and a significant weight increase in 8. There were no unfavorable side effects.

The authors emphasize that although the cause of anorexia should be determined so that the best treatment may be given, the empirical use of vitamin B₁₂ for anorexia may nevertheless be justified.

- 161 Masi, A. and Mori, S. Therapeutic activity of liver extracts and vitamin B₁₂ in children. *Minerva pediat* 5 290 April 30 1953 (abstr JAMA 153 1129 Nov 21 1953).

Liver extracts combined with vitamin B₁₂ were used to treat 30 children with acute respiratory and cardiovascular disease which tended to become chronic despite antibiotic, anti-infectious, and dietetic therapies. Atrophy, denutrition, asthenia, anorexia, and pallor were present in all these children. The 15 infants from

3 to 15 months old were given 1 Gm of liver extract and 15 mcg of vitamin B₁₂ orally every day for 20 to 25 days. The infants gained appetite and weight. Their general condition improved, and the number of erythrocytes increased as did levels of globulin and protein. Only a few under six months of age did not benefit. The 15 children from 2 to 15 years old received 2 Gm of liver extract and 15 mcg of vitamin B₁₂ intramuscularly every other day for 20 to 25 days. Changes in the blood were similar but superior to those in the younger group. Oral administration of vitamin B₁₂ alone produces only an increase of appetite and of weight when it is combined with liver extracts; there is also a slow but progressive improvement of the blood picture.

- 162 Lato M and Biscatti, O. Vitamin B₁₂ in chronic nutritional disturbances. *Lattante* 23 484 Aug 1952 (abstr AMA Am J Dis Child 86 215, Aug 1953).

Ten dystrophic infants were given 10 mcg of vitamin B₁₂ intramuscularly daily for 20 days. All gained weight and had an increase in the number of reticulocytes. Dystrophy is believed to be due to a conditioned or dietary deficiency of the B vitamins, especially of vitamin B₁₂.

- 163 DeMezel, M. B. The effect of vitamin B₁₂ in malnourished infants, *Arch argent de pediat* 37 31, 1952 (abstr AMA Am J Dis Child 86 674 Nov 1953).

A brief review of the pertinent literature in addition to observations in 7 controls and 8 patients leads the author to conclude that vitamin B₁₂ appears to have beneficial effects on weight gain in malnourished infants.

- 164 Karlin R. Variations du taux de vitamine B₁₂ dans le lait de femme Effet de l'administration de B₁₂ sur ce taux [Variations in the level of vitamin B₁₂ in mother's milk Effect of the administration of vitamin B₁₂ on these levels] *Presse méd* 63 1608 Nov 19 1955 (in Soc Proc)

Mother's milk is relatively poor in vitamin B₁₂. Only colostrum contains an amount approaching that of cow's milk. The quantity of vitamin B₁₂ diminishes considerably toward the fourth or fifth month of lactation (to 15 to 20 micrograms per 100 cc) and drops rapidly toward the end in some cases disappearing completely. The enrichment of maternal milk by oral or parenteral administration of vitamin B₁₂ to the mother is of interest in the treatment of disorders of growth in infants particularly in debilitated and premature infants. Ingestion of the vitamin alone gives variable and generally poor results but its ingestion with intrinsic factor permits a marked augmentation of vitamin B₁₂ levels in the milk. Intramuscular injection seems to be preferable however to oral administration it produces rapid and marked increases in the vitamin B₁₂ content of the milk lasting for 2 to 3 days.

- 165 Hofman I. La vitamina B₁₂ en altas dosis en la inapetencia infantil [Massive doses of vitamin B₁₂ for poor appetite in childhood] *Orientación méd* 2 1204-1210 Aug 27 1954

Of 674 children brought to the author because their mothers believed they had poor appetites only 10 had poor appetites for no discernible reason. Of these 10 9 had better appetites after receiving 5 to 10 doses of 1 000 mcg vitamin B₁₂ either intramuscularly or orally for 10 to 18 days. No undesirable side effects were encountered. The author emphasized that vitamin B₁₂ should not be given indiscriminately for anorexia as there is usually a

real cause for poor appetite which will respond to proper treatment.

- 166 Gouyen J and Bouvier R. Trial of a product combined vitamin B₁₂ and penicillin *Semaine d'hôp Paris* 31 975 977 March 14 1955 (abstr JAMA 158 612, June 18 1955)

Children from 3 months to 8 years of age suffering from weakness hypotrophy and anorexia or convalescing from illnesses were given a tablet containing 200 mcg of vitamin B₁₂ and 200 000 units of penicillin. No other therapy was given during the trial and no changes were made in the subjects' usual diet. The treatment was never given during an illness or immediately after an infection. Dosage generally ranged from a half tablet to two tablets daily. There were 18 weak hypotrophic anorectic children 2 who were convalescing from measles 3 from whooping cough 2 from infectious stomatitis and 5 from various other conditions. In evaluating the results of treatment the patient's weight curve appetite and appearance were considered. All but one child tolerated the product perfectly and two thirds were unquestionably improved in weight appetite and general condition. The rapidity of these gains in the convalescent group further indicated the value of vitamin B₁₂ and penicillin.

Paris France

- 167 Wokes F. Nutrition and vitamin B₁₂. *Lancet* 2 1343 Dec 24 1955 (Letter to Editor)

Study of data on vegan infants and children indicates that vitamin B₁₂ requirements tend to become more critical after early weaning. Under such circumstances the use of vitamin B₁₂ dietary supplements would seem to be a wise precaution. Letters from vegan parents whose children experienced vitamin B₁₂ deficiency fully justify this warning.

*Ovaltine Research Laboratories
King's Langley England*

- 168 Larcomb, J W, Perry, C S and Peterman R A Dietary supplementation of vitamin B₁₂ in prepuberty school age children 1 Growth studies *J Pediat* 45 70-74, July 1954

In a study of school age prepuberal children on a controlled institution diet 60 received a vitamin B₁₂ supplement of 20 mcg daily, 72 received a placebo and all 132 received a daily multiple vitamin supplement containing 1 mcg of vitamin B₁₂ activity

The results of this study confirmed earlier reports in that underweight children receiving vitamin B₁₂ showed statistically significant weight gains There was no change in the normal children but obese children showed a very slight weight gain No increase in height was seen in underweight or normal children but overweight children showed statistically significant height gain This height gain may suggest that vitamin B₁₂ participates in some fundamental metabolic growth regulating mechanism Data on nutritional signs have not yet been analyzed There was no outstanding beneficial effect in the cases of nerve deafness or optic nerve involvement

Ohio State University
College of Medicine
Columbus Ohio
536 Lake Shore Drive
Chicago Ill

- 169 Patrick, S J Some observations on the metabolism of vitamin B₁₂ by Jamaican children, *J Nutrition* 55 129-135, Jan 1955

A group of 19 Jamaican children on a low intake of animal protein received dietary vitamin B₁₂ supplementation 100 mcg daily for nine months Comparison of their plasma concentrations of vitamin B₁₂ during the last month of supplementation with those of a control group showed that the supplemented group had significantly higher plasma concentrations

(Plasma levels and urinary excretion of vitamin B₁₂ after a test dose of the vitamin were determined in 5 children with

retarded growth before and after dietary vitamin B₁₂ supplementation for nine months, and compared with the values obtained in 5 children with normal growth The latter showed higher plasma levels after the test dose than did the group with retarded growth but the differences were of doubtful significance The test dose caused consistently higher plasma levels in the retarded children after they had received the vitamin B₁₂ dietary supplementation No marked differences were observed between the groups in the percentage of the test dose excreted in the urine or in the rate of removal of the vitamin from the plasma

University College of the West Indies
Jamaica British West Indies

- 170 Campbell J A and McLaughlan J M Vitamin B₁₂ and the growth of children a review, *Canad M A J* 72 259 263, Feb 15, 1955

Authors summary Nine reports on the oral administration of vitamin B₁₂ to normal and underweight children and several reports on its use for chronically ill children have been reviewed The reports are conflicting and demonstrate above all, a need for critically controlled experiments before any definite conclusions can be reached regarding the possible effect of vitamin B₁₂ on growth in children To date available evidence indicates that the effect is far from spectacular and that there is little if any justification for the wide-spread use of vitamin B₁₂

Food and Drug Laboratories
National Health and Welfare
Ottawa Canada

- 171 Montoye H J Spata P J, Pinckney V and Barron L Effects of vitamin B₁₂ supplementation on physical fitness and growth of young boys *J Applied Physiol* 7 589 592, May 1955

This study was done as a preliminary step to determine whether supplementary vitamin B₁₂ in the diet helps to delay the onset of fatigue after strenuous exercise

The effect of adding 20 per cent protein to the basal diet in the absence of vitamin B₁₂ was similar to but less consistent than the effect observed with the high fat diets

*National Institutes of Health
Bethesda Md*

- 181 Richardson, L. R. Effect of large doses of vitamin B₁₂ on reproduction in rats, *Federation Proc* 13 475, March 1954

The effect of large quantities of vitamin B₁₂ on reproduction in rats was investigated with a synthetic diet which contained soybean protein (Drackett No 220) Two groups of 10 females each received 10 and 25 mcg, and two groups of 20 females each received 0 and 500 mcg of vitamin B₁₂/kg of diet, respectively Another group of 10 females received 100 mcg. of vitamin B₁₂/rat/wk by subcutaneous injection A female was given an opportunity to bear 4 litters and the number of young per litter was reduced to 8 at birth Only 58 per cent of the young observed in 73 litters which were born to females that received no vitamin B₁₂ were weaned while 88.1 to 96.1 per cent of young were weaned by those which received vitamin B₁₂ There was no evidence that more than 10 mcg was required or that larger quantities were toxic The females receiving vitamin B₁₂ by injection produced 39 litters and weaned 87.3 per cent of their young A sixth group of 8 females which had produced 4 litters while receiving vitamin B₁₂ at a level of 500 mcg were given 1000 mcg and allowed to bear additional litters Eighty five out of 86, or 98.8 per cent of the young were weaned These data show that large doses of vitamin B₁₂ are not injurious to reproduction *

*Texas Agricultural Experimental Station
College Station, Texas*

- 182 Wang, H., Scheid, H E and Schweigert, B S Histological studies with rats fed diets containing iodinated casein and different levels of vitamin

B₁₂, *Proc Soc Exper Biol. & Med.* 85 382-384, March 1954

Feeding of a vitamin B₁₂-deficient diet to rats caused degenerative changes in the thyroid gland, decreased spermatogenic activity, shrinkage of the seminiferous tubules and degeneration of interstitial tissues of the testes The thyroid changes were only partially counteracted by vitamin B₁₂ while the other symptoms subsided completely in response to vitamin B₁₂

*American Meat Institute Foundation, and
The University of Chicago
Chicago Ill*

- 183 Frölich, A. Relation between vitamin D and vitamin B₁₂, *Nature* 174 462-463, Sept 4, 1954

Diets containing high vitamin D supplements but no vitamin B₁₂ had a growth depressing effect on chicks. This was counteracted by vitamin B₁₂ supplements
*National Animal Experimental Station
Uppsala, Sweden*

- 184 Dryden, L P, Hartman, A. M. and Cary, C A Influence of vitamin B₁₂ upon vaginal patency in the rat, *Proc. Soc. Exper Biol & Med* 87 195 197, Oct 1954

Authors summary "The onset of sexual maturity in female rats as measured by vaginal patency, was found to be delayed in vitamin B₁₂-deficient animals as compared to vitamin B₁₂ supplemented littermates when their mothers were fed a B₁₂-deficient ration during lactation No such differences were observed when the mothers were fed B₁₂ during lactation. The nature of the carbohydrate and protein in the ration was found to have an effect on the differences observed

*U S Department of Agriculture
Beltsville Md*

- 185 Fatterpaker, P, Marfatia U and Sreenivasan A. Protective action of vitamin B₁₂ in the hyperthyroid rat, *Nature* 176 165, July 23, 1955

Four groups of rats were maintained on the following regimens (1) basal diet

alone (2) basal diet and vitamin B₁₂ (3) basal diet and iodinated casein (4) basal diet iodinated casein and vitamin B₁₂. The iodinated casein was given to induce hyperthyroidism and vitamin B₁₂ was administered to observe its effects on this condition. Percentage weight gains in the four groups during three weeks were as follows (1) 30.5 ± 7.4 (2) 11.2 ± 7.6 (3) -8.5 ± 2.3 (4) -1.8 ± 3.7 . Tests made after sacrifice of the animals showed that in the hyperthyroid animals there was a marked reduction in acetylating ability as well as in tissue glutathione content, which was counteracted by vitamin B₁₂ supplementation. From these results it is concluded that the primary manifestation of thyrotoxicosis is a deficiency of vitamin B₁₂.

*University of Bombay
Bombay India*

- 186 Ferguson T M., Rigdon R. H. and Couch J. R. A pathologic study of vitamin B₁₂-deficient chick embryos, A.M.A. Arch Path 60 393-400 Oct 1955

A pathologic study of 139 embryos obtained from vitamin B₁₂ depleted hens showed four main categories of lesions (1) decreased size (retarded growth) (2) edema and hemorrhages (3) necrotic foci in the liver brain and spinal cord and (4) increased fat in parenchymatous tissues.

*Texas Agricultural and
Mechanical College
College Station Texas
University of Texas Medical Branch
Galveston Texas*

- 187 Jones C C Brown S O Richard-
son, L. R. and Sinclair J G Tissue
abnormalities in newborn rats from
vitamin B₁₂ deficient mothers Proc
Soc Exper Biol & Med 90 135 140
Oct 1955

The authors conclude that when female rats are deprived of vitamin B₁₂ before mating until the end of gestation their progeny are weak and defective

Weight of the animals studied was below normal and development was proportionately arrested. The kidneys and heart were incompletely developed. The primitive reticular cells of the spleen were transformed into large densely chromatic and pyknotic cells. Vascular blocks with passive congestion especially in the liver were observed. The origin of the fat which blocked the sinusoids of the liver was not determined.

*Texas Agricultural and
Mechanical College
College Station Texas
University of Texas Medical Branch
Galveston Texas*

- 188 Whipple, G. H. Robschelt Robbins
F. S. and Bale W. F. Red cell stroma
protein rich in vitamin B₁₂ during
active regeneration. Anemia studies
using radioactive cobalt B₁₂ in dogs
J Exper Med 102 725 731, Dec. 1955

It appears that vitamin B₁₂ is an important factor in the early steps of stroma protein formation in the first few days of the life of the red cell in the dog.

*University of Rochester
Rochester N.Y.*

- 189 Richardson L. R. and Brock R.
Studies of reproduction in rats using
large doses of vitamin B₁₂ and highly
purified soybean proteins J Nutri-
tion 58 135 145 Jan 1956

Large doses of vitamin B₁₂ fed to mother rats were not toxic to the offspring. Moreover, the vitamin improved average weaning weight and increased the percentage of the litters that survived.

*Texas Agricultural Experiment Station
College Station Texas*

- 190 Jaffé W. O. Requirements of rats
for vitamin B₁₂ during growth repro-
duction and lactation J Nutrition
59 135 146 May 10 1956

Author's summary: A rat colony was kept on a fortified soybean oil meal-corn ration low in vitamin B₁₂ for 18 genera-

tions using mostly brother and sister matings. Litters starting with the second generation showed high mortality, low birth weights, low weaning weights, slow post weaning growth and low liver and kidney vitamin B₁₂ levels. Females of this group were older when giving birth to their first litters than the controls. Blood characteristics and liver glutathione levels were normal. No significant difference between succeeding generations could be detected and therefore no indication for a genetic selection toward resistance to vitamin B₁₂ deficiency could be found.

"The addition of 3 mcg of vitamin B₁₂ per kilogram of diet eliminated most of the deficiency symptoms but did not result in optimal weaning weights and post weaning growth while supplements of 5 mcg of vitamin B₁₂ per kilogram of diet, or 30 mcg of this vitamin together with 0.2% of methionine gave identical results in overcoming these deficiency signs. All of the animals used had been kept for at least one generation on the respective experimental diets previous to the experiments presented.

*Instituto Nacional de Nutrición
Caracas Venezuela*

- 191 Allen, S. H. The effects of vitamin B₁₂ deficiency and of copper deficiency on the concentration of free protoporphyrin in the erythrocytes of sheep, *Biochem J* 63 461-469, July 1956

In vitamin B₁₂-deficient sheep the concentrations of free protoporphyrin in the red blood cells are invariably much higher than normal. It increases more or less steadily as anemia develops.

*University of Adelaide
Adelaide Australia*

- 192 Ferguson T. M., Rigdon, R. H. and Couch J. R. Thyroid in B₁₂ deficient chick embryos. *Endocrinology* 60 13-21 Jan 1957

Authors summary "The histologic characteristics of the thyroid gland in vitamin B₁₂ deficient chick embryos are

described. The rate of development of the thyroid in the vitamin B₁₂ deficient embryos is 24 to 72 hours behind that of the normal chick embryo. The gland from the deficient embryo is larger, the follicles vary in size, and the amount of colloid is less than that present in the normal embryo. This study suggests that the thyroid from vitamin B₁₂ deficient embryos may be hypofunctional.

It was found that the vitamin B₁₂ content of eggs from hens fed an adequate diet ranged from 4 to 15 mcg per Gm of yolk while eggs from vitamin B₁₂-deficient hens contained 0.2 to 4 mcg per Gm of yolk.

*Texas Agricultural and
Mechanical College
College Station Tex
University of Texas Medical Branch
Galveston Texas*

- 193 Ferguson, T. M., Trunnell J. B., Dennis, B., Wade, P. and Couch J. R. The influence of vitamin B₁₂ deficiency on the uptake of I¹³¹ by the thyroid gland in adult and embryonic chickens, *Endocrinology* 60 28-32 Jan. 1957

Authors summary "The thyroid glands found in embryos from B₁₂-deficient hens exhibit reduced ability to concentrate I¹³¹. Moniodotyrosine, diiodotyrosine, thyroxine and iodide are present in the deficient glands as indicated by paper radio-chromatography.

The I¹³¹ uptake of pullets of hens fed a B₁₂-deficient diet may be somewhat increased by administration of vitamin B₁₂.

There is marked reduction bordering upon absence of sulfhydryl groups in the thyroid glands of vitamin B₁₂ deficient embryos and hens.

*Texas Agricultural and
Mechanical College
College Station Texas
University of Texas
Galveston Texas
M. D. Anderson Hospital and
Tumor Institute
Houston Texas*

- 194 Zarrow M X Horger L M and McCarthy J L Atrophy of adrenal gland following thiouracil and vitamin B₁₂ Proc. Soc. Exper Biol & Med 94 348-349 Feb 1957

Thiouracil administration causes growth inhibition and adrenal atrophy in rats. In this experiment rats were given vitamin B₁₂ 10 or 20 mcg daily intraperitoneally for 12 weeks along with the oral thiouracil. Vitamin B₁₂ prevented growth inhibition but failed to prevent adrenal atrophy. Thus adrenal atrophy following administration of thiouracil is not a reflection of generalized growth inhibition but must be attributable to a specific action of thiouracil on the adrenal.

Purdue University
Lafayette Ind

NUTRITION IN THE ELDERLY

- 195 Chow B F Vitamin B₁₂ and aging Federation Proc 13 453-454 March 1954

In studies comparing the absorption and disposition of vitamin B₁₂ administered orally and parenterally to 8 young persons (20 to 40 years) and to 18 old subjects (60 to 90 years) the author notes several significant differences:

- (1) Old persons retain a larger portion of injected vitamin B₁₂ than do the young.
- (2) Samples of gastric juice from old subjects who did not have achlorhydria contained less vitamin B₁₂ binding substance than similar samples from the young group.
- (3) The mean level of vitamin B₁₂ serum of the old group was significantly lower than that of the young.
- (4) Oral tolerance tests showed that all 8 young subjects in comparison with only 11 of the 18 old subjects after re-

ceiving 10 mg vitamin B₁₂ orally responded with a marked elevation in blood level. After the test dose was lowered to 250 mcg, none of the 10 old subjects showed an increase, however 5 of the 8 young persons responded with an elevation of the blood level.

These studies indicate that because of lack of intrinsic factor older people are less able to absorb exogenous vitamin B₁₂ and thus the vitamin B₁₂ level is lowered in blood and tissues.

Johns Hopkins University
Baltimore Md

- 196 Chow B F The absorption of vitamins and enzymic activities in aging Internat Rec Med 167 429-430 July 1954 (in Soc Proc)

A test for determining absorption and utilization of vitamin B₁₂ consists of obtaining a fasting blood sample, then feeding a large dose of vitamin B₁₂, withdrawing two additional blood samples 1½ and 3 hours later, and analyzing all the blood samples for vitamin B₁₂ content. The normal level of vitamin B₁₂ in young individuals was approximately 250 micrograms, and there was a 90 per cent response to a 1 mg dose of oral vitamin B₁₂ in 10 out of 11 young subjects.

Of 36 people averaging 70 years of age, a 40 per cent average response occurred in 14 after a similar dose. Old people have a lowered vitamin B₁₂ binding capacity, generally reflected by their increased tendency toward achlorhydria; perhaps they have a lowered content of intrinsic factor available for binding vitamin B₁₂. When vitamin B₁₂ is injected in old individuals, more of it is retained than in the young. This is interpreted to mean that there is less tissue saturation in old subjects; hence they retain more of the injected vitamin.

Johns Hopkins University
Baltimore Md

- 197 Boger, W P, Wright, L D, Strickland, S C, Gylfe, J S and Cumnera, J L Vitamin B₁₂ correlation of serum concentrations and age, *Proc Soc Exper Biol & Med* 89 375-378, July 1955

Authors summary 'Total serum vitamin B₁₂ concentrations were determined in 528 individuals by the *L. leichmannii* method, and a justification for the choice of this method of assay is presented. The individuals studied were normal as far as physical health is concerned, and the major variable was age. In such persons there is a trend toward lower serum concentration of vitamin B₁₂ in the aged than in younger age groups. Although this difference is statistically significant, the biological importance of this finding is not apparent. There is wide individual difference of values in serum vitamin B₁₂ concentration at all age levels but in this normal group values below 200 µmcg/ml and above 1000 µmcg/ml were uncommon. The average for the entire 528 individuals was 560 µmcg/ml, the 95% confidence bands being 70 and 1060 µmcg/ml respectively.

Norristown State Hospital
Norristown, Pa

Sharp & Dohme
Division of Merck & Co Inc
West Point, Pa

- 198 Monat, H A Nutrition of aged cardiac patients, *Geriatrics* 10 581-582 Dec 1955

The author states that the majority of aged cardiac patients are in a deplorable nutritional state and that the various cardiac drugs reduce the desire for food. He recommends a diet allowing 20 calories, 1 Gm of protein and 0.5 Gm of fat per Kg of body weight, the rest being allotted to carbohydrates. Necessary daily dietary adjuncts include calcium, iron, 25,000 I.U. of vitamin A, 10 mg of thiamine, 10 mg of riboflavin, 16 mg of niacin, 5 mcg of vitamin B₁₂, 2 mg of folic acid, 150 mg of ascorbic acid and 1,000

I.U. of vitamin D. In addition, when the patient is first placed on the above regimen, daily intramuscular injections of 30 mcg of vitamin B₁₂ and 100 mg of thiamine should be given for 30 days or until definite improvement is evident. The author comments: 'Paradoxically this calorically submaintenance diet in a short time improves the patient's general physiologic processes.'

Washington, D.C.

- 199 Zintel, H A Nutritional rehabilitation of the elderly patient undergoing surgery, *Am J Clin Nutrition* 3 501-510 Nov-Dec 1955

The writer believes that all elderly patients need supplementary vitamins especially after surgical operation or protracted illness. He states that vitamin B₁₂ is a potent antianemic substance and is important in transmethylation, and in the utilization of marginal intakes of protein. He states: 'It is well to remember that (1) the clinical avitaminoses are rare; (2) the subclinical deficiencies are common especially in the aged; (3) the subclinical deficiencies have multiple and profound influences on the physiologic processes of the body; and (4) for optimum therapy the physician must assume that all old elderly patients are deficient.'

St. Luke's Hospital
New York, N.Y.

- 200 Kaufman, W The use of vitamin therapy to reverse certain concomitants of aging, *J Am. Geriatrics Soc* 3 927-936, Nov 1955

In 35 patients over 45 years of age, none of whom had tropical sprue or pernicious anemia, the constant symptoms were mild dysequilibrium, impaired memory and concentration, depression, insomnia, nervous irritability and fatigue. Less constant symptoms consisted of breathlessness with unpaired heart sounds, dyspepsia, paresthesias, and difficulty in controlling the bladder. These symptoms

disappeared within two weeks after the patients received intramuscular injections of 100 mcg. of vitamin B₁₂ once or twice a week. After three months of this therapy some patients had recurrences within a week after treatment was discontinued but reinstitution of therapy alleviated the symptoms. Most patients receiving this treatment remained well for three to nine months.

Bridgeport, Conn.

- 201 Glass G B J, Goldbloom A A, Boyd L J, Laughton R, Rosen S and Rich, M. Intestinal absorption and hepatic uptake of radioactive vitamin B₁₂ in various age groups and the effect of intrinsic factor preparations. *Am J Clin Nutrition* 4 124 133 March April 1956

Measurement of hepatic uptake of 0.5 mcg. of Co⁶⁰ vitamin B₁₂ orally administered to 60 individuals from 18 to 90 who were normal or had some irrelevant disorder indicates wide variation in the intestinal absorption of vitamin B₁₂.

There is no evidence of general impairment of intestinal absorption of vitamin B₁₂ as a function of advancing age but more older than younger individuals have impaired intestinal absorption of vitamin B₁₂.

In individuals over 60 absorption is significantly lower in subjects with gastric hypo- or acidity than in those with normal or hyperacid gastric secretion. Measurement of the hepatic uptake of Co⁶⁰ vitamin B₁₂ might be helpful in the early recognition of vitamin B₁₂ absorption defects of a sprue-like or pernicious anemia-like type in this group.

Addition of a potent intrinsic factor preparation increases hepatic uptake of radioactive vitamin B₁₂ over 25 per cent in almost half the subjects in all age groups. Especially important is the increased hepatic uptake in older individuals with decreased gastric acidity which is usually associated with reduction of other gastric secretions.

Intrinsic factor preparations appear helpful not only in states of frank vitamin B₁₂ deficiency such as pernicious anemia or total gastrectomy but also in clinically latent partial defects of absorption.

New York Medical College
Flower and Fifth Avenue Hospitals and
Bird S. Coler Memorial Hospital and Home
New York, N.Y.

- 202 Chow B F, Wood R, Horonick A and Okuda K. Age-wise variation of vitamin B₁₂ serum levels. *J Gerontol* 11 142 146 April 1956

Serum vitamin B₁₂ levels were measured in young and old individuals and in rats by means of the microbiologic method of Skeggs and Wright. Preliminary studies revealed that vitamin B₁₂ serum levels were not influenced by dietary intake immediately preceding the drawing of blood samples and were relatively constant in the same individuals measured at different times.

Study A. There were 210 subjects: 65 young (age 25 to 35) students, technical assistants or prisoners and 145 old individuals (prisoners, inmates of homes for the aged and members of a club who lived at home). The young subjects had definitely higher serum vitamin B₁₂ levels than the old ones. For example, the mean vitamin B₁₂ value in mcg. per cc. of serum for young students and technicians was 231.5 ± 27.6 while that of a group in an old age home was 151.8 ± 18.7 .

Study B. In this group there were 114 healthy subjects ranging in age from 10 to over 70 selected at random from the Baltimore population. Again the vitamin B₁₂ content of serum definitely decreased with advancing age.

Study C. The findings in human beings were confirmed in rats. Both male and female rats showed statistically significant decreases in serum vitamin B₁₂ levels with advancing age.

The authors conclude that the advisability of administering vitamin B₁₂ to certain old individuals in order to bring

the serum level of vitamin B₁₂ to normal needs further study since the clinical effect of this measure has not been determined

*Johns Hopkins University
Baltimore Md*

*Geriatric Institute of the
Daughters of Jacob
Bronx NY*

- 203 Blumberg N and Zisserman L. Diabetes in the older age group. *Geriatrics* 11 444-446, Oct 1956

In an article devoted primarily to diabetes in the aged the authors refer to studies now in progress aimed at revealing physiologic aspects of vitamin B₁₂ in the aged. It is stated that there is a relatively low serum level of vitamin B₁₂ in aged individuals as a group and that glucose tolerance seems to be directly related to the vitamin B₁₂ serum level. The exact significance of these findings is not yet clear.

*Home for the Jewish Aged
Philadelphia Pa*

- 204 Gaffney G W, Horonick A, Okuda K, Meier P, Chow, B F and Shock, N W. Vitamin B₁₂ serum concentrations in 528 apparently healthy human subjects of ages 12 to 94, *J Gerontol* 12 32-38 Jan 1957

Serum vitamin B₁₂ levels were determined in healthy subjects ranging in age from 12 to 94. Of the 528 subjects 89 were in patients of a gerontology branch of Baltimore City Hospital, 55 were physicians or staff members of the hospital ranging in age from 20 to 49, 97 were male prison inmates receiving an adequate diet, 161 were individuals from the Baltimore population and 126 were healthy residents or staff members of a private home for the aged.

Blood was withdrawn in the morning from fasting patients and its vitamin B₁₂ content was assayed by a microbiologic method. An almost uniform gradual decline in mean serum vitamin B₁₂ levels was noted with advancing age in all

groups. Low serum vitamin B₁₂ levels were found more frequently in persons over 50 years old than in younger persons. There was no substantial difference in serum vitamin B₁₂ levels of men and women.

The authors conclude. Available evidence supports the contention that observed regression of serum B₁₂ levels on age is statistically significant. The agreement in regression lines between the several groups, widely different in background and living conditions, suggests that the B₁₂ level is relatively unaffected by these conditions. If dietary conditions per se play a significant role in the age wise decrease in serum B₁₂ in the groups studied, they do so in consequence of an age dependent alteration in the selection of foods by the subjects themselves.

*National Institutes of Health
Bethesda Md*

*Johns Hopkins University and
Baltimore City Hospitals
Baltimore Md*

*Geriatric Institute of the
Daughters of Jacob
Bronx NY*

- 205 Tauber S A, Goodhart R M, Hsu J M, Blumberg N, Kassab, J and Chow, B F. Vitamin B₁₂ deficiency in the aged, *Geriatrics* 12 368-374, June 1957

Serum levels of vitamin B₁₂ and the glutathione content of erythrocytes were determined in 20 young and 21 old healthy individuals. The serum vitamin B₁₂ levels of the old people were significantly lower than those of the young people: 160 ± 14.5 and 297 ± 17.7 micrograms per cc respectively. Likewise the glutathione content of the erythrocytes in the old individuals was significantly lower than in the young people. It has been shown that erythrocyte glutathione levels are low in vitamin B₁₂ deficiency in rats and man. These findings thus provide additional evidence of the existence of a state of vitamin B₁₂ undernutrition in the aged.

the mother. In 89 of 96 (92 per cent) of the cases the infant's vitamin B₁₂ concentration was higher than that of the mother the differences being in some cases as much as sixfold in favor of the infant. A large number, 28 of 96 (29 per cent) of the umbilical-cord serum values were above 1060 micromicrogram per milliliter, which has been defined as the upper band of 95 per cent confidence limits for 'normal adults'. This seeming avidity of the infant for vitamin B₁₂ has not been explained.

The lowered serum vitamin B₁₂ concentration due to pregnancy was confirmed. Occasional unexplained high values for serum vitamin B₁₂ were again observed (vitamin administration was eliminated as a possible cause).

'The importance of determining normal values concurrently in the same institution from which abnormal groups are derived is emphasized.

*Norristown State Hospital
Norristown, Pa.*

- 210 Baker, H., Erdberg, R., Pasher, I. and Sobotka, H. Study of folic acid and vitamin B₁₂ in blood and urine during normal pregnancy, *Proc Soc Exper Biol & Med* 94 513-515, March 1957.

Blood and urine levels of vitamin B₁₂ and folic acid were determined in 47 women during pregnancy. Vitamin preparations free of vitamin B₁₂ and folic acid were given but the women were not on a controlled diet. Assays of vitamin B₁₂ used *Euglena gracilis* and *Ochromonas malhamensis*; assays of folic acid used a thermophilic bacillus.

The median urinary vitamin B₁₂ level was 55 micromicrograms per cc; the median blood level was 193 micromicrograms per cc. Moreover the majority of the urinary levels were below 60 micromicrograms per cc and one third of the blood levels were below 125 micromicrograms per cc. These median values are

two to five times lower than normal for urine and four times lower for blood. The low serum vitamin B₁₂ levels observed as pregnancy progressed suggest that the fetus may attract maternal vitamin B₁₂ across the placenta, thereby depleting this vitamin in the maternal circulation.

The median level of folic acid in urine and blood was 19 micromicrograms per cc, two to eight times as high as normal. The authors state that high maternal stores of vitamin B₁₂ and folic acid during pregnancy should alleviate some of the stress which fetal development places upon maternal metabolism and could prevent intrauterine injury.

*MT Sinai Hospital
New York, N.Y.*

- 211 Hellegers, A., Okuda, K., Nesbitt, R. E. L., Jr., Smith, D. W. and Chow, B. F. Vitamin B₁₂ absorption in pregnancy and in the newborn, *Am J Clin Nutrition* 5 327-331, May-June 1957.

Absorption of vitamin B₁₂ was measured in pregnant and nonpregnant women and rats. Thyroid activity and serum vitamin B₁₂ levels were studied in 2 children with cretinism.

Oral doses of 1000 mcg, 500 mcg and 250 mcg were given to pregnant and nonpregnant women and the serum vitamin B₁₂ levels were measured before administration and 90 minutes and three hours afterward. A positive response (increase of 160 mcg or more) occurred in 8 of 9 pregnant subjects but in only 2 of 7 nonpregnant women at the higher dose. Differences between the two groups using the lower dosages were not statistically significant.

Serum vitamin B₁₂ levels were measured in normal rats before mating and two days before delivery. The vitamin B₁₂ serum level decreased to about half its original value during pregnancy. Absorption was also studied in rats before mating and near delivery and in nonpregnant

controls A dose of 50 micromicrograms of radioactive vitamin B₁₂ was given orally followed by injection of the same amount of unlabelled vitamin B₁₂ two hours later Radioactivity of the urine feces and fetus was measured The pregnant animals showed about a twofold increase in absorption and 60 per cent or more of the absorbed vitamin was concentrated in the fetus Co⁶⁰ vitamin B₁₂ 10 micromicrograms was injected into pregnant and nonpregnant rats Assay of liver kidney and fetus showed that the target organs of pregnant animals contained much less radioactivity than those of controls and the absorbed vitamin B₁₂ was concentrated in the fetus

The authors have reported (in press) that in spite of low maternal serum vitamin B₁₂ levels, the fetus could show adequate or high serum levels They have seen 2 instances in which neither mother nor fetus showed appreciable serum levels of vitamin B₁₂ and the latter showed cretinism The mother's serum vitamin B₁₂ level returned to normal without thyroid or vitamin B₁₂ administration, but the infant's serum vitamin B₁₂ level did not return to normal until thyroid therapy was given, and then the rise was rapid The authors suggest that the fetal serum vitamin B₁₂ level rather than the maternal level is significant in cretinism Further studies on cretin infants showed that the administration of thyroid extract to cretins will rapidly lead to return of the vitamin B₁₂ levels to normal

*Johns Hopkins University
Baltimore 6 Md*

METABOLIC STUDIES IN HUMANS

- 212 Editorial Vitamin B₁₂ research J A M.A. 153 960 Nov 7, 1953

At a meeting of scientists from many fields evidence was presented that indicates an important role of the vitamin

[B₁₂] in the synthesis of nucleic acids and in the formation or function of such metabolically active compounds as glycine serine methionine and choline Interrelationships between vitamin B₁₂ and other vitamins such as pyridoxine and pantothenic acid were demonstrated Additional evidence for the interdependence of vitamin B₁₂ and the folic acid series was presented and the role of intrinsic factor was reviewed Growth studies were reported emphasizing the importance of adequate supplies of the vitamin in the metabolism of carbohydrate and fat, including not only the conversion of carbohydrates to fat but the metabolism of fat itself

Vitamin B₁₂ has been shown to play an important role in modifying the concentration of sulfhydryl-containing compounds of physiological importance The hypothesis was presented that in the absence of adequate supplies of the vitamin coenzyme A would not be reduced from the disulfide to the sulfhydryl form and would therefore inhibit the normal pathways of fat metabolism

- 213 Kaye R, Caughey R H and McCrory W W The minimal nitrogen requirement of infants and the effect of vitamin B₁₂ upon it A M.A. Am J Dis Child 86 326-327 Sept 1953 (in Soc Proc)

Experiments to determine the minimal nitrogen requirement for equilibrium in 5 boy babies showed that, on an average intake of 98 mg of nitrogen per Kg the nitrogen retention averaged -2 mg per Kg per day and the average weight gain was 1 Gm per Kg per day the 2 patients given more than 100 mg per Kg per day were in positive balance Three similar subjects given an average nitrogen intake of 870 mg per Kg per day showed an average nitrogen retention of 269 mg per Kg per day and a weight gain of 13 Gm per Kg per day

Oral doses of 30 to 300 mcg of vita

mun B₁₂ per day did not increase nitrogen retention in the high protein subjects in fact, the average retention decreased from 269 to 213 mg a day but as this represented only 5 per cent of the intake it was not considered significant. In the low protein group vitamin B₁₂ increased average nitrogen retention from 3 to 20 mg per Kg per day but this was of questionable significance because most of the increase was accounted for by one infant. Changes in rate of weight gain were in the same direction as changes in nitrogen balance.

Vitamin B₁₂ administration was associated with an increase of 25 per cent in urinary nitrogen excretion. Animal experiments of others suggest that this might be a reflection of an enhanced conversion of amino acids into fat or carbohydrate.

Philadelphia Pa

- 214 Kaye R, Caughey R. H and McCrory W W Effects of vitamin B₁₂ and Aureomycin on nitrogen retention in infants. *Pediatrics* 13 462-475, May 1954

The effects of vitamin B₁₂ on weight, and on nitrogen and electrolyte balances were studied in 6 male infants. Five appeared to have made subnormal growth progress and 1 was recovering from Leiner's disease. Three of the infants were offered nitrogen intakes of approximately 10 Gm per Kg per day and the remaining 3 an intake of 0.1 Gm per Kg per day.

The authors were unable to obtain unequivocal evidence that vitamin B₁₂ exerted a nitrogen anabolic effect in these infants. Those on a high protein intake had an increased urinary nitrogen excretion which may have reflected an enhanced rate of conversion of protein into carbohydrate or fat. Averages of the control and vitamin B₁₂ period nitrogen retentions and weight gains in Gm per Kg per day were 0.235 and 11.8 for those fed high protein diets and 0.11 and 2.4 for those fed low protein diets.

Appetite stimulation was noted in 2 of the 6 subjects given B₁₂.

Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine Philadelphia Pa

- 215 Wokes F and Picard C W The role of vitamin B₁₂ in human nutrition, *Am J Clin Nutrition* 3 383-390 Sept-Oct 1955

Hypotheses of the role of vitamin B₁₂ in metabolic systems are formulated. The theories involve the assumption that hydroxocobalamin (or aquocobalamin) rather than cyanocobalamin is the active form of vitamin B₁₂. The presence of cobalt in an organic coordination complex permits cyanide to be taken up by the vitamin. The authors present a hypothetical scheme of cyanide utilization which suggests a mechanism for increased thiocyanate excretion in deficient subjects and offers an explanation of the methionine or choline sparing action of vitamin B₁₂. Either methionine or cysteine could act as sulfur donors in this system. A hypothetical sulfur cycle is outlined which the authors believe occurs in the liver.

Ovaltine Research Laboratories King's Langley England

METABOLIC STUDIES IN ANIMALS

- 216 Doctor, V M Welch B E, Perrett R W Brown C L, Gabay, S and Couch J R Metabolic interrelationship between folic acid, vitamin B₁₂ and the citrovorum factor. *Proc Soc Exper Biol & Med* 84 29-32 Oct 1953

Supplementing a low vitamin B₁₂ PGA (pteroylglutamic acid) chick diet either with increasing levels of vitamin B₁₂ or increasing levels of PGA resulted in a greater capacity by the liver homogenates to convert added PGA to CF (citrovorum factor).

Texas Agricultural Experiment Station College Station Texas

- 217 Firth, J Mistry S P James M F
and Johnson B C. Vitamin B₁₂ and
transmethylation in the baby pig. *Proc.*
Soc. Exper Biol & Med 85 307 309
Feb 1954

The effect of a vitamin B₁₂ deficiency
on transmethylation from methionine to
choline was studied in the baby pig. The
deficiency did not influence the level of
choline in the liver nor the excretion of
urinary choline. Histologic examination
of livers and kidneys did not reveal the
fatty infiltration or renal damage charac-
teristic of a choline deficiency. This study
indicated that vitamin B₁₂ is not involved
in this direct transmethylation.

University of Illinois
Urbana, Ill

- 18 Vitamin B₁₂ and methyl group syn-
thesis. *Nutrition Rev* 12 218 220
July 1954

The literature on the influence of vita-
min B₁₂ on the synthesis of methyl
groups is reviewed. It appears that vita-
min B₁₂ reduces the absolute dietary re-
quirements for the methyl group appar-
ently by promoting the biosynthesis of
this group.

- 219 Fatterpaker P Marfatia U and
Sreenivasan A. Role of folic acid
and vitamin B₁₂ in transmethylation.
Part I. Formation of creatine in vitro
and in vivo. *Indian J M Research*
43 43 50 1955 (abstr. *Blood* 11 685
July 1956)

Deficiency of either folic acid or vita-
min B₁₂ or both led to a diminution in
creatine formation and excretion. The
effect was especially seen in vitro syn-
thesis in vivo it was less pronounced in
the urine and least in skeletal muscle. On
the administration of large doses of folic
acid or vitamin B₁₂ to the deficient ani-
mals there was rapid improvement in the
impaired creatine metabolism. In con-
trast addition of folic acid or vitamin B₁₂

did not activate in vitro the creatine
forming enzymes in the deficient systems
Univ. coll. of Bombay
Bombay India

- 220 Verly, W G and Cathey W J. The
influence of vitamin B₁₂ on the bio-
synthesis of the methyl group of cho-
line from methanol. *J Biol Chem*
213 621-624 April 1955

Methyl synthesis from C¹⁴ methanol
in rats deficient in vitamin B₁₂ and doubly
deficient in vitamin B₁₂ and folic acid was
compared with that in vitamin B₁₂ treated
controls. The deficient animals incor-
porated less isotope into the methyl group
of choline than the controls. Animals de-
ficient in folic acid as well as vitamin B₁₂
showed less incorporation than animals
deficient in vitamin B₁₂ alone.

Cornell University
Ithaca N Y

- 221 Boxer, G E Shonk C E Gullilan
E W, Emerson G A and Ozinsky
E L. Changes in coenzyme A con-
centration during vitamin B₁₂ defi-
ciency. *Arch. Biochem & Biophys*
59 24 32 Nov 1955

A striking increase in coenzyme A
(CoA) concentration in the liver of vita-
min B₁₂-deficient chicks has been prelimi-
narily reported. These data needed con-
firmation in another species. Data on the
CoA concentration in the liver and other
organs of vitamin B₁₂-deficient rats have
therefore been obtained and the state of
reduction of the increased CoA in liver
has been determined.

Of the 49 rats used 20 received diets
deficient in vitamin B₁₂ 29 received vita-
min B₁₂ supplements and 10 in each of
the two groups also received thyroid
powder.

The CoA concentration in the liver
and kidney of vitamin B₁₂-deficient male
and female rats is two to three times
higher than in controls. This increase is
independent of the added stress of thy-
roid powder feeding.

The increased CoA in the catalytically active reduced form. The rate of degradation of CoA is the same in homogenates of livers from deficient and normal animals.

Merck & Co. Inc.
Rahway N.J.

- 222 Wong, W. T. and Schweigert, B. S. Role of vitamin B₁₂ in nucleic acid metabolism. II. Liver coenzyme A levels in the rat. *Arch. Biochem. & Biophys.* 60: 126-129, Jan. 1956.

The present studies were designed to determine the coenzyme A (CoA) levels in livers of vitamin B₁₂-deficient and supplemented rats to provide further information on the role of this vitamin in metabolism.

Some weanling rats from mothers fed diets deficient in vitamin B₁₂ and casein were fed similar diets for six weeks, while others received supplements of vitamin B₁₂. After sacrifice the CoA content of their livers was determined.

The deficient groups showed a definitely smaller weight gain and other abnormalities characterized by a lower content of liver nucleic acids. The CoA level was two to three times greater in the livers of vitamin B₁₂ deficient animals than in those of the controls. No overlapping in the range of values was noted.

American Meat Institute Foundation and
University of Chicago
Chicago III

- 223 Wong, W. T. and Schweigert, B. S. Role of vitamin B₁₂ in nucleic acid metabolism. I. Hemoglobin and liver nucleic acid levels in the rat. *J. Nutrition* 58: 231-242, Feb. 10, 1956.

Authors' summary: "The growth rate, liver DNA [deoxyribonucleic acid] and RNA [ribonucleic acid] levels, and liver regeneration ability after partial hepatectomy of rats fed an [0.06%] iodinated casein basal diet deficient in vitamin B₁₂

were lower than those of supplemented controls.

In subsequent experiments an even more complicated vitamin B₁₂ deficiency was produced in rats born from mothers fed the vitamin B₁₂-deficient diet without iodinated casein during gestation and lactation. The growth rate and liver nucleic acid composition of these rats fed the basal ration were significantly lower than those for the controls fed supplemented rations. A syndrome characterized by presence of porphyrin whiskers and scaly feet was also noted in the deficient rats.

The addition of dried liver to the non iodinated casein ration did not improve the growth promoting activity of vitamin B₁₂ when the latter was present at a level of 50 mcg./kg. ration. However, inclusion of 4 or 10% of dried liver in the diet of the female rats, and 10% of liver in the diet of male rats significantly increased the hemoglobin content of the blood, as compared with that observed when the vitamin B₁₂-deficient diet was fed. The significance and implications of these findings were discussed.

University of Chicago and
American Meat Institute Foundation
Chicago III

- 224 Register, U. D. Effect of vitamin B₁₂ on liver and blood nonprotein sulfhydryl compounds. *J. Biol. Chem.* 206: 705, 1954 (abstr. *Am. J. Clin. Nutrition* 2: 373, Sept. Oct. 1954).

Weanling rats born to mothers maintained on vitamin B₁₂ deficient diets were continued on the deficient diets, and their weight gains, liver and blood values for ergothioneine, glutathione, and total nonprotein sulfhydryl groups were compared with those in animals receiving the same diet plus injections of vitamin B₁₂. There was a marked decrease, largely due to glutathione, in the levels of liver and blood sulfhydryl groups in the deficient animals. There was no significant effect on the liver or blood ergothioneine.

- 225 Ling C. T. and Chow, B. F. The influence of vitamin B₁₂ on carbohydrate and lipid metabolism J Biol Chem. 206 797-805 Feb 1954 (abstr Blood 9 930 Sept 1954)

In vitamin B₁₂-deficient rats glucose tolerance tests produced abnormally high blood sugar levels. Such rats consistently showed lower blood sugar levels after short periods of fasting but developed hunger diabetes sooner than control animals. The phospholipid content of the body tissues of such deficient rats was much less per unit weight than the phospholipid content in the tissues of vitamin B₁₂-treated rats. In two patients with pernicious anemia in relapse the phospholipid content of the blood was low but rose in treatment with B₁₂. A high carbohydrate-low fat diet supplemented with injections of glucose solution produced in normal rats not only a hyperglycemia but also concomitant decrease in glutathione content of the blood. Administration of glutathione produced transient but significant drops in the blood sugar of these animals. As vitamin B₁₂-deficiency tends to lower blood glutathione it is suggested that the maintenance of an adequate concentration of glutathione may be one of the roles of vitamin B₁₂. It is suggested also that glutathione may be involved in the utilization of carbohydrate. Thus it is concluded that vitamin B₁₂ plays an important role in carbohydrate and lipid metabolism and that its effect on blood glutathione concentration may be of significance in its role in metabolism.

*Johns Hopkins University
School of Hygiene and Public Health
Baltimore Md*

- 226 Ling C. T. and Chow B. F. Effect of vitamin B₁₂ on ribose formation in erythrocytes Federation Proc 13 253 March 1954

Our previous studies indicate that (1) vitamin B₁₂-deficiency results in impaired carbohydrate metabolism and decreased blood glutathione concentration

and (2) an interrelationship exists between glucose utilization and glutathione concentration. Several investigators have presented evidence implicating vitamin B₁₂ in the synthesis of nucleosides or nucleic acids. It has been found that whereas thymidine and other desoxyribosides or desoxyribonucleic acid may replace vitamin B₁₂ for the growth of certain microorganisms thymine cannot. Since thymine differs from thymidine in that it lacks desoxyribose, vitamin B₁₂ may be concerned with the sugar moiety of the nucleosides or nucleic acids. The influence of this vitamin on carbohydrate metabolism and nucleic acid synthesis could be readily comprehensible on this basis. In this connection, it should be interesting to ascertain whether vitamin B₁₂ influences the formation of ribose in tissue cells. The first phase of this study concerns the *in vitro* formation of ribose in erythrocytes with glucose as substrate. Incubation of erythrocytes in isotonic phosphate buffer containing glucose under aerobic conditions for 2 to 3 hours resulted in a marked increase of ribose in the erythrocytes. These results suggest that ribose formation may be an important pathway of glucose utilization in mammalian erythrocytes. Red cells from B₁₂-deficient rats showed significant depression of ribose formation and oxygen consumption as compared with erythrocytes from B₁₂-repleted or normal animals. Addition of reduced glutathione to the incubation mixture restored the normal rate of ribose synthesis and oxygen consumption.

*Jefferson Medical College
Philadelphia Pa*

*Johns Hopkins School of Hygiene and
Public Health
Baltimore Md*

- 227 Meites J. and Feng Y. S. L. Counteraction by vitamin B₁₂ of protein catabolic effects of cortisone. Federation Proc 13 468-469 March 1954

Some investigations have indicated that large doses of vitamin B₁₂ partially

The increased CoA is in the catalytically active reduced form. The rate of degradation of CoA is the same in homogenates of livers from deficient and normal animals.

*Merck & Co. Inc.
Rahway N J*

- 222 Wong W T and Schweigert, B S
Role of vitamin B₁₂ in nucleic acid metabolism. II Liver coenzyme A levels in the rat, *Arch Biochem & Biophys* 60 126-129, Jan 1956

The present studies were designed to determine the coenzyme A (CoA) levels in livers of vitamin B₁₂-deficient and supplemented rats to provide further information on the role of this vitamin in metabolism.

Some weanling rats from mothers fed diets deficient in vitamin B₁₂ and casein were fed similar diets for six weeks while others received supplements of vitamin B₁₂. After sacrifice, the CoA content of their livers was determined.

The deficient groups showed a definitely smaller weight gain, and other abnormalities characterized by a lower content of liver nucleic acids. The CoA level was two to three times greater in the livers of vitamin B₁₂ deficient animals than in those of the controls. No overlapping in the range of values was noted.

*American Meat Institute Foundation, and
University of Chicago
Chicago Ill*

- 223 Wong W T and Schweigert B S
Role of vitamin B₁₂ in nucleic acid metabolism. I Hemoglobin and liver nucleic acid levels in the rat. *J Nutrition* 58 231-242, Feb 10, 1956

Authors summary The growth rate, liver DNA [desoxyribonucleic acid] and RNA [ribonucleic acid] levels and liver regeneration ability after partial hepatectomy of rats fed an [0.06%] iodinated casein basal diet deficient in vitamin B₁₂

were lower than those of supplemented controls.

In subsequent experiments an uncomplicated vitamin B₁₂ deficiency was produced in rats born from mothers fed the vitamin B₁₂-deficient diet without iodinated casein during gestation and lactation. The growth rate and liver nucleic acid composition of these rats fed the basal ration were significantly lower than those for the controls fed supplemented rations. A syndrome characterized by presence of porphyrin whiskers and scaly feet was also noted in the deficient rats.

The addition of dried liver to the non iodinated casein ration did not improve the growth promoting activity of vitamin B₁₂ when the latter was present at a level of 50 mcg/kg ration. However, inclusion of 4 or 10% of dried liver in the diet of the female rats and 10% of liver in the diet of male rats, significantly increased the hemoglobin content of the blood as compared with that observed when the vitamin B₁₂-deficient diet was fed. The significance and implications of these findings were discussed.

*University of Chicago and
American Meat Institute Foundation
Chicago Ill*

- 224 Register U D Effect of vitamin B₁₂ on liver and blood nonprotein sulfhydryl compounds, *J Biol Chem* 206 705, 1954 (abstr. *Am J Clin Nutrition* 2 373 Sept-Oct 1954)

Weanling rats born to mothers maintained on vitamin B₁₂-deficient diets were continued on the deficient diets and their weight gains, liver and blood values for ergothioneine, glutathione, and total nonprotein sulfhydryl groups were compared with those in animals receiving the same diet plus injections of vitamin B₁₂. There was a marked decrease largely due to glutathione in the levels of liver and blood sulfhydryl groups in the deficient animals. There was no significant effect on the liver or blood ergothioneine.

- 225 Ling C. T and Chow B F The influence of vitamin B₁₂ on carbohydrate and lipid metabolism J Biol Chem. 206 797-805 Feb 1954 (abstr Blood 9 930 Sept 1954)

In vitamin B₁₂-deficient rats glucose tolerance tests produced abnormally high blood sugar levels. Such rats consistently showed lower blood sugar levels after short periods of fasting but developed hunger diabetes sooner than control animals. The phospholipid content of the body tissues of such deficient rats was much less per unit weight than the phospholipid content in the tissues of vitamin B₁₂-treated rats. In two patients with pernicious anemia in relapse the phospholipid content of the blood was low but rose in treatment with B₁₂. A high carbohydrate-low fat diet supplemented with injections of glucose solution produced in normal rats not only a hyperglycemia but also concomitant decrease in glutathione content of the blood. Administration of glutathione produced transient but significant drops in the blood sugar of these animals. As vitamin B₁₂-deficiency tends to lower blood glutathione it is suggested that the maintenance of an adequate concentration of glutathione may be one of the roles of vitamin B₁₂. It is suggested also that glutathione may be involved in the utilization of carbohydrate. Thus it is concluded that vitamin B₁₂ plays an important role in carbohydrate and lipid metabolism and that its effect on blood glutathione concentration may be of significance in its role in metabolism.

*J. Am. Hopkins University
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Baltimore Md*

- 226 Ling C T and Chow B F Effect of vitamin B₁₂ on ribose formation in erythrocytes Federation Proc 13 253 March 1954

Our previous studies indicate that (1) vitamin B₁₂-deficiency results in impaired carbohydrate metabolism and decreased blood glutathione concentration

and (2) an interrelationship exists between glucose utilization and glutathione concentration. Several investigators have presented evidence implicating vitamin B₁₂ in the synthesis of nucleosides or nucleic acids. It has been found that whereas thymidine and other desoxyribosides or desoxyribonucleic acid may replace vitamin B₁₂ for the growth of certain microorganisms thymine cannot. Since thymine differs from thymidine in that it lacks desoxyribose, vitamin B₁₂ may be concerned with the sugar moiety of the nucleosides or nucleic acids. The influence of this vitamin on carbohydrate metabolism and nucleic acid synthesis could be readily comprehensible on this basis. In this connection it should be interesting to ascertain whether vitamin B₁₂ influences the formation of ribose in tissue cells. The first phase of this study concerns the *in vitro* formation of ribose in erythrocytes with glucose as substrate. Incubation of erythrocytes in isotonic phosphate buffer containing glucose under aerobic conditions for 2 to 3 hours resulted in a marked increase of ribose in the erythrocytes. These results suggest that ribose formation may be an important pathway of glucose utilization in mammalian erythrocytes. Red cells from B₁₂-deficient rats showed significant depression of ribose formation and oxygen consumption as compared with erythrocytes from B₁₂-repleted or normal animals. Addition of reduced glutathione to the incubation mixture restored the normal rate of ribose synthesis and oxygen consumption.

*Jefferson Medical College
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Baltimore Md*

- 227 Meites J and Feng Y S L. Counteraction by vitamin B₁₂ of protein catabolic effects of cortisone. Federation Proc 13 468-469 March 1954

Some investigations have indicated that large doses of vitamin B₁₂ partially

overcome the ability of cortisone to inhibit body hair and thymus growth in young rats fed diets deficient in the vitamin and it has been suggested that the latter may enhance the availability of protein to the organism. To test this hypothesis a vitamin B₁₂-deficient diet was fed to rats. After 20 or 30 days they were given 200 mcg of vitamin B₁₂ per Kg of diet alone or with increasing amounts of cortisone acetate for three successive 10-day periods. Cortisone was also administered alone. Vitamin B₁₂ alone increased body weight and food intake but did not alter daily urinary nitrogen losses. Cortisone alone greatly increased urinary nitrogen losses and reduced growth and food intake. Vitamin B₁₂ completely counteracted these actions of cortisone under ad libitum feeding but was ineffective when food intake was limited to that of rats given cortisone without vitamin B₁₂. The authors conclude that vitamin B₁₂ may be unable to alter normal urinary nitrogen values but can prevent a cortisone-induced increase in nitrogen losses by increasing appetite and consequently the intake of carbohydrate and protein.

Michigan State College
East Lansing, Mich

- 228 Aschkenasy, M. A. La vitamine B₁₂ et le métabolisme des protéines [Vitamin B₁₂ and the metabolism of proteins] *Presse méd* 62 1148, Aug 14, 1954

Vitamin B₁₂ is not only an erythropoietic substance but also a polyvalent factor taking part in protein metabolism and probably also in fat and carbohydrate metabolism.

The vitamin is indispensable to the growth of young animals and to the maintenance of weight of adult animals. It improves nitrogen balance favoring certain tissues and organs (blood, liver, lymphoid organs) more than others.

It is difficult to produce avitaminosis B₁₂ in animals because of (1) large tissue

reserves of this vitamin (2) its synthesis by intestinal microflora and (3) the possibility of its partial replacement by methionine.

Vitamin B₁₂ seems to have its maximum effects on growth in subjects who are undernourished or on diets deficient in animal protein. Therefore deficiencies of both vitamin B₁₂ and methionine may be found in the same subject. One of the reasons why antibiotics promote growth is that they promote intestinal synthesis of vitamin B₁₂.

Study of the anabolic action of vitamin B₁₂ shows that it is extremely complex. This factor participates in enzymatic reactions effecting synthesis and in the transfer of labile methyl groups. The vitamin also has an important role, in conjunction with folic and folinic acids in the synthesis of nucleosides, nucleic acids and nucleoproteids, and it also takes part in the formation of porphyrins and in the metabolism of sulphhydryl compounds.

Paris, France

- 229 Charkey, L. W., Kano, A. K. and Anderson, J. A. Effects of fasting on free amino acid levels in chick blood as modified by vitamin B₁₂. *J Biol Chem* 210 627-632 Oct 1954

Authors summary A study of fasting in chicks in the presence and absence of dietary vitamin B₁₂ has shown the following:

1 A fasting rise in free amino acid in blood is associated with metabolic unavailability of the amino acid precursors.

2 Fasting effects on the levels of free amino acids in blood are functions of the age of the experimental chicks up to 6 weeks of age.

3 Of five amino acids studied, lysine shows the most and leucine the least extensive and enduring (in terms of chick age) rise in blood level due to fasting.

4 Vitamin B₁₂ functions as a moderator of fasting effects on blood levels of free amino acids. This moderating action

is operative in a range of vitamin B₁₂ nutritional levels above that required for maximal growth

*Col ad Agricultural and
Mechanical College
Fort Collins Colo*

- 230 Chang I And Johnson B C The effect of vitamin B₁₂ on some aspects of glycine metabolism Arch Biochem & Biophys 55 151 156 March 1955

The addition of vitamin B₁₂ to the diet caused a five-fold increase in incorporation of the α -carbon of glycine into the methyl group of choline while vitamin B₁₂ supplementation had no effect on the incorporation of the α -carbon of glycine into serine and aminooethanol

*University of Illinois
Urbana Ill*

- 231 Wagle S R. and Johnson B C Role of vitamin B₁₂ in nucleic acid synthesis Federation Proc 16 401 March 1957

It is well established that formate glycine serine and the methyl group of methionine serve as precursors in the biosynthesis of purines and pyrimidines which are then incorporated into RNA and DNA. Vitamin B₁₂ has been postulated to be involved in this synthesis. Formate-C¹⁴, formaldehyde C¹⁴, glycine 2-C¹⁴, serine 3-C¹⁴ and methionine-methyl C¹⁴ were injected into vitamin B₁₂-deficient and B₁₂ normal chicks and baby pigs. Similarly glucose-C¹⁴ uniformly labeled was injected into deficient and normal baby pigs. Animals were killed after 4 hours. Both RNA and DNA were isolated from the livers and the radioactivity was determined. The total radioactivity of the RNA and of the DNA isolated from the formate and formaldehyde injected animals was higher than that of the RNA and DNA isolated from the animals which received the other precursors (glycine serine and methionine). However in no case was any difference

found between B₁₂-deficient and the B₁₂ normal animals. Similarly no difference in activity was found in RNA and DNA isolated from the livers of the pigs (both deficient and normal) injected with radioactive glucose. These results suggest that vitamin B₁₂ is neither involved directly in the incorporation of 1-carbon precursors into nucleic acids in the conversion of glucose to ribose, nor in the incorporation of ribose into nucleic acids. The results obtained were similar for both pigs and chicks.

*University of Illinois
Urbana Ill*

- 232 Sure H Effect of amino acid and vitamin B₁₂ supplements on the biologic value of proteins in rice and wheat J Am Dietet A 31 1232 1234 Dec 1955

Experiments were carried out on rats to determine the biologic value of the proteins in polished rice processed polished rice and enriched hard wheat flour when vitamin B₁₂ and amino acids were included in the diet. The only source of protein was the rice or wheat. The diet included roughage wheat germ cod liver oils hydrogenated vegetable shortening glucose thiamine riboflavin pyridoxine niacin calcium pantothenate para-aminobenzoic acid inositol and choline chloride. The amino acids added were L-lysine DL-threonine and DL-methionine.

The most significant observation in this investigation is the remarkable influence of minute doses of vitamin B₁₂ when supplemented with amino acids which are deficient in the proteins of milled wheat flour on growth and efficiency of protein utilization. Vitamin B₁₂ in addition to stimulating greater food intake also produces greater efficiency of protein utilization as evidenced from gains in body weight per gram of food intake. Such data suggest that vitamin B₁₂ may participate as a coenzyme or activator of intestinal peptidases.

thus making more essential amino acids available during digestion for protein synthesis *

*University of Arkansas
Fayetteville Ark*

- 233 Kik, M C Nutritional improvement of rice, *J Am Dietet A* 32 647-650, July 1956

Supplements of lysine threonine and vitamin B₁₂ were added to whole milled, and enriched rice, and growth of young rats on this diet was studied. The addition of lysine and threonine to their rations produced an average gain of 117.2 Gm. and a protein efficiency ratio of 2.61 Gm. When vitamin B₁₂ (0.1 mcg) was added, there was an additional increase in weight and protein efficiency ratio.

*University of Arkansas
Fayetteville Ark*

- 234 Henry, K M and Kon, S K Vitamin B₁₂ and protein metabolism, *Brit J Nutrition* 10 39 1956 (abstr *J Am Dietet A* 32 658, July 1956)

'The effect of vitamin B₁₂ on protein metabolism has been studied in a series of experiments with B₁₂-deficient and normal rats. For the deficient rats the biologic value of casein was increased to the same extent by the addition of 1 per cent methionine as by the addition of 1 per cent homocysteine and a vitamin B₁₂ supplement. Homocysteine was ineffective in the absence of B₁₂. It is concluded that vitamin B₁₂ is involved in the synthesis of methyl groups.

METABOLIC DISTURBANCES (ENDOCRINE)

- 235 Mettes, J and Feng, Y S L Effects of insulin on vitamin B₁₂ requirements *Federation Proc.* 14 100 March 1955

'1) On a vitamin B₁₂ deficient diet single injections of insulin reduced blood sugar only about 1/2 as much in normal and alloxan diabetic rats and only about

1/3 as much in cortisone injected rats as in similarly treated animals fed a vitamin B₁₂ abundant diet. 2) Insulin injections greatly reduced the urinary excretion of tracer doses of radioactive vitamin B₁₂ in normal alloxanized and cortisone-treated rats whether on a vitamin B₁₂-deficient or abundant ration. Insulin was least effective in this respect in the cortisone treated vitamin B₁₂-deficient rats. 3) In glucose tolerance tests (750 mg injected intraperitoneally, followed by blood glucose determinations 1 and 2 hours later), increases in blood and urinary glucose were at least 7 fold greater in rats fed the vitamin B₁₂-deficient than in rats fed the vitamin B₁₂ abundant diet. These values were about twice as high in alloxanized and cortisone-injected rats fed the former as compared to the latter diet. 4) These results suggest that a) insulin increases requirements for vitamin B₁₂ in normal, alloxan-diabetic and most of all in cortisone treated rats. b) vitamin B₁₂ is essential for normal carbohydrate metabolism confirming the work of Chow et al (1951 54) and c) the action of large doses of cortisone in increasing vitamin B₁₂ needs (Mettes et al 1951 54) is mediated in part through the pancreas.'

*Michigan State College
East Lansing Mich*

- 236 Best, W R Leithold, S and David D Urinary excretion of vitamin B₁₂ Co⁵⁷ in myxedema *J Lab & Clin Med* 48 783, Nov 1956 (in Soc. Proc.)

Urinary excretion of Co⁵⁷ vitamin B₁₂ was studied in a man with marked megaloblastic anemia and myxedema to elicit possible relationships between these diseases. Response to vitamin B₁₂ therapy had been subnormal. Doses of 1.0 or 2.0 mcg of Co⁵⁷ vitamin B₁₂ with or without 1 to 3 USP units of intrinsic factor concentrate orally were given, followed by 1.0 mg vitamin B₁₂ parenterally, the percentage of Co⁵⁷ vitamin B₁₂ in the 24-hour urine was then measured. Four sets of tests made during nine months of thyroid therapy (mostly 120 mg per day) showed

low or low intermediate excretions (under 4 per cent or 6 per cent with 10 mcg) Co⁵⁷ vitamin B₁₂ excretion remained low after four days of tetracycline

Two other patients with untreated myxedema showed low or low intermediate Co⁵⁷ vitamin B₁₂ and intrinsic factor concentrate excretions. One received chlorotetracycline therapy for four days and the intrinsic factor concentrate test remained low. Three other myxedema patients, one under treatment, showed normal excretions (over 8 per cent with 10 mcg).

Megaloblastic anemia in hypothyroidism is rare and has been attributed to chance. Tests in 3 hypothyroid patients suggest that, in some cases, absorption of vitamin B₁₂ is hampered by intestinal block or by intraluminal inactivation of intrinsic factor or of vitamin B₁₂. Poor intrinsic factor activity indicates poor secretion or an inactivating substance in the patient's gastric juice. Inhibition was demonstrated in one of three aspirates. This plus paradoxical and prolonged 72 hour excretions suggests variable regurgitation from the intestine of an inhibitor which delays absorption. This inhibitor is probably nonbacterial as antibiotics failed to correct malabsorption. Constant malabsorption despite prolonged therapy could indicate inadequate dosage of thyroid, irreversible change resulting from hypothyroidism or a hypothyroid state secondary to intestinal malfunction.

Chicago III
Hlaes III

- 237 Lavate, W V and Sreenivasan A. Protective action of vitamin B₁₂ in the hyperthyroid rat, *Nature* 178: 804-805, Oct 13 1956

In the hyperthyroid rat there is a marked fall in the concentration of vitamin B₁₂ in the circulating blood and in the percentage retained in the blood cells; this is prevented by vitamin B₁₂ administration.

University of Bombay
Bombay India

- 238 Ralli E D, Barbosa F X., Dumm, M E., Beck, E M and Laken, B. Prolonged administration of vitamin B₁₂, folic acid and calcium pantothenate in patients with diabetes mellitus. *J Clin Endocrinol & Metab* 15: 898 July 1955 (in Soc. Proc.)

Diabetic patients under observation for several years were the subjects of this study. Vitamin B₁₂, vitamin B₁₂ plus folic acid or calcium pantothenate was administered to study the effects on the course of the disease, on the insulin requirement and on blood constituents. Vitamin B₁₂ did not cause striking change in the plasma or nonprotein sulfhydryl levels (glutathione). Some patients however showed an increased sensitivity to insulin. The administration of calcium pantothenate was in some cases associated with a decrease in the fasting levels of blood glucose.

New York University
Bellevue Medical Center
New York N Y

OTHER CLINICAL AND EXPERIMENTAL USES

OPHTHALMIC CONDITIONS

- 239 Becker B, Lang C A and Chow, R F. Vitamin B₁₂ excretion and diabetic retinopathy, *Am J Clin Nutrition* 1: 417-423 Sept-Oct 1953

Urinary excretion of vitamin B₁₂ was studied in patients with diabetes mellitus, some of whom had retinopathy. An intra-

muscular injection of 50 mcg or 65 mcg was given and the urine was assayed microbiologically for vitamin B₁₂. Several patients received radioactive vitamin B₁₂ and their urine was measured for radioactivity.

Diabetic patients with retinopathy excreted significantly more (average 19 mcg) vitamin B₁₂ than nondiabetic sub-

jects (average 9.6 mcg) while diabetic patients without retinopathy excreted even less (average 4.2 mcg) than healthy controls. Administration of testosterone decreased excretion of vitamin B₁₂ in all diabetics with retinopathy who were tested. (Normal humans and diabetics without retinopathy were not studied.)

A correlation has been noted between Kimmelstiel-Wilson lesions of the kidney and diabetic retinopathy. Perhaps the difference in vitamin B₁₂ excretion by diabetics with and without retinopathy is due to differences in renal function.

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School of Hygiene and Public Health
The Wilmer Institute and
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Baltimore Md*

Chow, B F. Vitamin B₁₂ and diabetic retinopathy, *Mod Med* 21: 195 Nov 15, 1953 (in Late Reports from Medical Centers)

Blindness with advanced diabetes and old age may be related to inability to absorb vitamin B₁₂. Measurement of urinary excretion of the vitamin after administration of radioactive doses showed that diabetic patients with retinopathy excreted about 19 mcg, those without eye lesions 4.2 mcg, and healthy subjects 9.6 mcg. The patients with early retinitis showed definite need of vitamin B₁₂.

*Johns Hopkins University
Baltimore Md*

Rifkin, H. The Kimmelstiel-Wilson syndrome and its variants. *New York State J Med* 53: 2947-2950 Dec 15, 1953

This article reviews the clinical features, pathogenesis, diagnosis, and treatment of the disease. The author mentions clinical and experimental evidence indicating that relative adrenal hyperfunction, possibly associated with vitamin B₁₂ deficiency, may be related to the development of both glomerular and retinal lesions.

*Montefiore Hospital
New York N Y*

242 Chow, B F, Rosen, D A and Lang C A. Vitamin B₁₂ serum levels and diabetic retinopathy, *Proc Soc Exper Biol & Med* 87: 38-39, Oct 1954

Serum levels of vitamin B₁₂ were compared in diabetic patients, 16 with and 16 without diabetic retinopathy. The patients with retinal lesions had much higher serum vitamin B₁₂ levels than diabetics without retinal involvement: 292 ± 24 micrograms per cc and 162 ± 18 micrograms per cc, respectively.

It is stated that diabetics with retinopathy apparently handle vitamin B₁₂ differently from those who do not have the complication and that vitamin B₁₂ is involved in the pathogenesis of the retinopathy. The authors mention data which indicate that the hyperglycemia and glycosuria induced by prolonged injection of cortisone can be corrected by the parental administration of vitamin B₁₂. These and other data lead to the suggestion that administration of vitamin B₁₂ with insulin, cortisone, or ACTH might offer clinical advantages over the administration of these hormones alone.

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Johns Hopkins Hospital
Baltimore Md*

243 Bellavia M and Pellegrino, F. Vitamin B₁₂ in ophthalmology, *Arch Otol* 56: 65-82, Jan-Feb 1952 (abstr *Am J Ophth* 36: 1160 Aug 1953)

Vitamin B₁₂ was used locally and systemically in the treatment of corneal lesions of rabbits. The authors report that lesions healed 25 per cent faster in treated animals than in the controls.

A case of fascicular keratitis which showed no response to penicillin, vitamin D₂, and atropine, cleared after vitamin B₁₂ had been administered locally and systemically for eight days.

Eczematous keratitis in 5 patients and corneal abscess in 1 also showed satisfactory improvement after treatment with vitamin B₁₂.

- 244 Bellavia M Vitamin B₁₂ in high doses in some corneal diseases Arch ophthalmol 59 517-523 Nov-Dec 1955 (abstr Am J Ophthalmol 41 724, April 1956)

Intractable dystrophic or paralytic conditions of the cornea in 6 patients were effectively treated with local applications of a solution of vitamin B₁₂ (500 mcg vitamin B₁₂ per cc)

OSTEOARTHRITIS

- 245 Melendez E O Junemann B C and Berríos R Acción de la vitamina B₁₂ en las artrosis [Effect of vitamin B₁₂ in arthritis] Rev méd de Chile 73 76 Aug 1953 (reprint Semana méd Supl Diar 3130D Jan 6 1954)

Of 32 patients with radiologically confirmed osteoarthritis treated with one to five weekly 100 mcg intramuscular injections of vitamin B₁₂ 21 made good improvement 6 satisfactory improvement and 5 showed poor results Of the 27 with favorable results 22 were still well a month later 5 received only temporary relief Twenty-eight experienced an increase in general well being and appetite There were no hematologic changes in the 8 patients studied for them

None of these patients had other rheumatic conditions and any treatment other than vitamin B₁₂ had been discontinued for at least a month before vitamin B₁₂ was given

- 246 Norcross H M Lockie L M and Talbott J H Osteoarthritis Gen Practitioner 11 93-101 March 1955

The authors discuss the pathology differential diagnosis and treatment of osteoarthritis and the means used for relief of pain and swelling Their experience shows that weekly doses of 1 000 mcg of

vitamin B₁₂ are effective when symptoms are radicufar in origin

University of Buffalo and
Buffalo General Hospital
Buffalo N Y

SERUM LEVELS

- 247 Unglaub W G Rosenthal H L and Goldsmith G A Studies of vitamin B₁₂ in serum and urine following oral and parenteral administration J Lab & Clin Med 43 143-156 Jan 1954

Comparisons of vitamin B₁₂ activity in serum and urine as indicated by microbiologic assays using *L. leichmannii* were made after oral and parenteral administration of the vitamin The authors conclude Urine vitamin B₁₂ activity following oral doses of 500 or 1 000 mcg differed little from that prior to administration of the vitamin Following doses of 3 000 mcg an increase in urine vitamin B₁₂ activity equivalent to that which followed intramuscular injection of 10 mcg of the vitamin was noted in most instances No definite relationship between maximum total serum vitamin B₁₂ activity and urinary excretion of the vitamin was apparent Maximum levels of total serum vitamin B₁₂ activity and urinary excretion tended to increase with repeated oral doses of the vitamin No correlation was observed between maximum total serum vitamin B₁₂ activity and hematopoietic response

Louisiana University School of Medicine and
Charity Hospital of Louisiana
New Orleans La

- 248 Pitney W R and Beard M F Serum and urine concentrations of vitamin B₁₂ following oral administration of the vitamin Am J Clin Nutrition 2 89-96, March-April 1954

The concentration of vitamin B₁₂ in the serum of normal subjects and of pa-

tients with pernicious anemia, after doses of 1 000 and 5 000 mcg was determined by a method which distinguishes between free vitamin B₁₂ and that bound in a heat labile complex with serum protein

The oral administration of 1 000 mcg produced no significant change but with 5 000 mcg both normal subjects and pernicious anemia patients showed increased serum concentrations In patients with pernicious anemia there was a correlation between the finding of vitamin B₁₂ in the serum and the hematologic response The authors results confirm other reports that the vitamin B₁₂ serum levels are reduced in pernicious anemia It is suggested on the basis of experimental results that the critical serum level necessary for the maintenance of normal erythropoiesis is more than 20 micromicrograms per cc

Vitamin B₁₂ administered orally is absorbed from the intestine in bound form and appears in the serum in a different form than when it is given intramuscularly Thus it is impractical to estimate the amount absorbed after oral administration by comparing the amount excreted in the urine with that excreted after smaller amounts given intramuscularly It appears that bound vitamin B₁₂ is not excreted in the urine whereas free vitamin B₁₂ is so excreted It is suggested that orally administered vitamin B₁₂ raises the serum level of bound vitamin B₁₂ and does not contribute to free vitamin B₁₂ levels to the extent that parenteral vitamin B₁₂ does

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School of Medicine
Louisville Ky

- 249 Pitney W R, Beard, M F and Van Loon, E J Observations on the bound form of vitamin B₁₂ in human serum, J Biol Chem 207 143 152, April 1954 (abstr Blood 11 1208 Dec 1954)

The vitamin B₁₂ normally present in human serum is combined with protein primarily It is split off, to a certain extent

at least, when serum proteins are denatured by heat A microbiological assay technic employing *Euglena gracilis* distinguishes between free and bound vitamin B₁₂ As parenteral injection of more than 80 micrograms of B₁₂ has been followed by the rapid urinary excretion of the major part of the dose it has been suggested that there is a limit to the capacity of serum to bind the vitamin Proteins of normal sera were fractionated by paper strip electrophoresis and the vitamin B₁₂ concentration measured by microbiological assay Normally most, if not all of the B₁₂ was bound to the alpha globulins The addition of crystalline B₁₂ to normal sera revealed a limited capacity to bind the vitamin Consistently however the total amount of vitamin recovered by bioassay did not equal the amount added to the sera This raises the question of whether some technical refinement is needed or whether there is in serum a substance that inhibits the activity of B₁₂ in this particular type of assay It is possible megaloblastic anemia may result from a deficiency in vitamin B₁₂ binding protein Further studies on correlation of vitamin B₁₂ and alpha globulin concentration in sera will be of interest

University of Louisville
School of Medicine and
Veterans Administration Hospital
Louisville Ky

- 250 Ross, G I M, Mollin D L, Cox E V and Ungley, C C Hematologic responses and concentration of vitamin B₁₂ in serum and urine following oral administration of vitamin B₁₂ without intrinsic factor, Blood 9 473 488, May 1954

Serum and urine vitamin B₁₂ levels (determined by microbiological assay) have been correlated with hematologic effect in pernicious anemia patients receiving vitamin B₁₂ by mouth or (in a few cases) rectally Observations were made in 38 patients with pernicious anemia in 2 with megaloblastic anemia following partial gastrectomy and in 4 normal sub-

jects The oral doses ranged from 500 to 3 000 mcg the rectal dose was 1 000 or 3 000 mcg of vitamin B₁₂ given in 500 cc of saline

The response to the 500 mcg oral dose of vitamin B₁₂ was variable in all subjects With oral doses of 1 000 mcg or 3 000 mcg all patients showed some hematologic response This variable response was associated with a variable rise in serum concentration of vitamin B₁₂ The average hematologic effect of 3 000 mcg of vitamin B₁₂ by mouth in the pernicious anemia patients was equivalent to that produced by 40 mcg of vitamin B₁₂ given intramuscularly The average response to an oral dose of 1 000 mcg was about equivalent to that following 20 mcg intramuscularly

In 14 of 16 patients in whom excretion studies were done the urinary excretion of the vitamin increased after doses of 1 000 or 3 000 mcg of vitamin B₁₂ but there was no obvious correlation between the hematologic effect of the dose and the amount of vitamin B₁₂ excreted in the first 24 hours The average amount of the vitamin excreted in the urine after the oral doses of 3 000 mcg was only one quarter of the amount excreted after the injection of 40 mcg This lower excretion appears due to the lower serum concentrations of the vitamin and particularly to the lower serum concentration of uncombined vitamin In 3 of 4 normal subjects after doses of 3 000 mcg by mouth there was a rise in vitamin B₁₂ levels of both serum and urine

Rectal administration of vitamin B₁₂ resulted in adequate hematologic response in all 4 patients receiving 3 000 mcg and in 1 of 3 receiving 1 000 mcg Evidence of absorption (increased serum vitamin B₁₂ levels) was obtained in most of these patients

These studies show that intrinsic factor plays little part in absorption of large oral doses of vitamin B₁₂ This view is supported by the fact that the average increase in the serum vitamin B₁₂ concen-

tration of the normal subjects given 3 000 mcg by mouth was no greater and in fact was slightly less than that of the patients with pernicious anemia Moreover, very large amounts of normal gastric juice would be required even for the absorption of smaller quantities such as 30 to 40 mcg Finally doses of 1 000 or 3 000 mcg of vitamin B₁₂ given by rectum where presumably no intrinsic factor was present resulted in similar hematologic effects and changes in serum vitamin B₁₂ concentrations

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251 Mollin D L and Ross G I M
Vitamin B₁₂ deficiency in the megaloblastic anaemias Proc Roy Soc Med
47 428-431, June 1954

This study presents the results of assays of vitamin B₁₂ in the serum of more than 280 patients with various types of megaloblastic anemia Patients with pernicious anemia subacute combined degeneration of the cord total or partial gastrectomy or intestinal anastomosis had low serum levels of vitamin B₁₂ Normal levels were found in patients with idiopathic steatorrhea nontropical nutritional megaloblastic anemia and megaloblastic anemia of pregnancy

Vitamin B₁₂ given intramuscularly or orally (in small doses with intrinsic factor or in large doses without it) raised the vitamin B₁₂ serum levels of patients with pernicious anemia to within the normal range The values remained elevated for varying periods depending on the size of the dose the amount excreted and on the individual requirements of the patient for vitamin B₁₂

The authors conclude that the assay of serum vitamin B₁₂ levels (a more sensitive method than ordinary hematologic techniques) is useful in detecting vitamin B₁₂ deficiency in gastrectomized patients in vegetarians or in relatives of patients with pernicious anemia Study of such

groups should indicate how soon anemia develops after the serum vitamin B₁₂ level has fallen below the normal range. In some patients with pernicious anemia the serum vitamin B₁₂ level has been low but the blood picture normal or almost normal for at least one to two years. Serum level assay is also a most convenient method of determining the specific vitamin deficiency in patients with megaloblastic anemia.

*Postgraduate Medical School of London
London, England*

- 252 Beard, M F Pitney W R and Sanneman E H. Serum concentrations of vitamin B₁₂ in patients suffering from leukemia. *Blood* 9:789-794, Aug 1954.

The vitamin B₁₂ concentrations of serum were determined in patients with various types of leukemia. In 22 normal subjects vitamin B₁₂ serum concentrations varied from 86 to 460 micromicrograms per cc. In 18 patients with chronic lymphatic leukemia the mean of 209 micromicrograms per cc was close to that of the normals. In 12 patients with chronic myeloid leukemia the serum vitamin B₁₂ levels were about 15 times greater than the mean of the normals. The range for the myeloid leukemia group was from 540 to 6,500 micromicrograms per cc. Of 7 patients with acute leukemia 3 had levels of vitamin B₁₂ in serum within the normal range and 4 had moderately elevated levels (range 216 to 1,000 micromicrograms per cc, mean 599 micromicrograms per cc).

The present study devoted mainly to sera from patients with chronic myeloid leukemia, showed that vitamin B₁₂ was totally in the bound form. Electrophoretic studies of sera from 3 patients with myeloid leukemia showed that alpha globulin fractions were the main source of bound vitamin B₁₂. This is also the case in normal sera but the binding capacity of this fraction in myeloid leukemic sera is much higher. Remissions in myeloid

leukemia were accompanied by a fall in serum vitamin B₁₂ concentrations but these remained well above normal. During relapses a rise in vitamin B₁₂ concentrations in serum occurred. In general high white cell counts in myeloid leukemia were associated with higher levels of vitamin B₁₂ in the serum.

It is postulated (a) that the high serum levels of vitamin B₁₂ in myeloid leukemia may be associated with myeloid activity (b) that in the disease alpha globulin is qualitatively different in its ability to bind the vitamin and (c) that the high vitamin B₁₂ levels may reflect decreased erythroid activity.

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School of Medicine
Louisville, Ky*

- 253 Beard, M F Pitney W R, Sanneman E H, Sakol, M J and Moorhead H H. Serum concentrations of vitamin B₁₂ in acute leukemia. *Ann Int Med* 41:323-327, Aug 1954.

Serum levels of vitamin B₁₂ were determined in 56 normal persons and in 20 patients with various types of acute leukemia. Patients with acute lymphocytic leukemia had normal vitamin B₁₂ serum concentrations; those with acute myelocytic leukemia had mean concentrations of about 13 times normal and the patients with acute monocytic leukemia had moderately elevated levels. There was no correlation in any of the leukemia types between the serum vitamin B₁₂ concentration and the total white cell count.

In the discussion the authors comment. *In vitro* normal serum will bind only from 203 to 576 $\mu\text{mcg/ml}$ of vitamin B₁₂ when excess free vitamin is added. In the cases of acute myelocytic leukemia studied the mean concentration of bound vitamin was found to be 2,570 $\mu\text{mcg/ml}$. The results demonstrate that the serum protein responsible for vitamin B₁₂ binding is abnormal in acute myelocytic leukemia. It is possible that the binding protein is liberated into the serum by the disintegration of myeloid

cells. The lack of correlation between the serum vitamin B₁₂ concentrations and white cell counts would make this appear unlikely. Our results suggest that vitamin B₁₂ therapy would be of no avail in the treatment of acute leukemia as in no case were serum concentrations found below normal.

Louisville Ky

- 254 Lear A A, Harris J W, Castle W B and Fleming E M. The serum vitamin B₁₂ concentration in pernicious anemia. J Lab & Clin Med 44 715-722 Nov 1954

The results of microbiologic assays of serum vitamin B₁₂ levels using *Euglena gracilis* are reported. The mean total serum vitamin B₁₂ level in 20 normal subjects was 532 micromicrograms per cc all had free vitamin B₁₂ present. In 33 patients with pernicious anemia in relapse the mean level was 39 micromicrograms per cc. and no free vitamin B₁₂ was detected. Twelve patients with megaloblastic anemia that subsequently responded to folic acid therapy all had levels within the normal range and a mean level of 307 micromicrograms per cc.

In 5 patients with hepatic cirrhosis the mean vitamin B₁₂ serum level was 714 micromicrograms per cc. most of which was in bound form. None had received therapy with vitamin B₁₂. It is possible that the serum of these patients possesses increased vitamin B₁₂ binding capacity since the globulins are characteristically elevated and abnormal in such patients.

Harvard Medical School and Boston City Hospital
Boston, Ma

- 255 Wilson H E and Pitney W R. Serum concentrations of vitamin B₁₂ in normal and nutritionally deficient monkeys. J Lab & Clin Med 45 590-598 April 1955

The vitamin B₁₂ serum levels of monkeys fed a diet deficient in vitamin B₁₂

and folic acid showed a gradual decline. Addition of folic acid and 2 mcg of vitamin B₁₂ caused little change but 10 or 20 mcg of vitamin B₁₂ caused a rise to high normal levels.

Animals with severe folic acid deficiency showed normal vitamin B₁₂ levels which increased during recovery from the folic acid deficiency. In animals with ascorbic acid deficiency low vitamin B₁₂ levels were seen. The vitamin B₁₂ rose to a high level during recovery from this deficiency. The authors state that ascorbic acid may be influential in absorption of vitamin B₁₂ and folic acid. These studies also indicated that the monkey is able to store a considerable amount of vitamin B₁₂. The comparison between the serum vitamin B₁₂ concentrations in monkeys and in man is not conclusive since greater amounts of the free vitamin are found in monkey serum than in human serum.

Northwestern University Medical School
Evanston Ill
University of Louisville
Louisville Ky

- 256 Lear A A. Effect of folic acid on serum vitamin B₁₂ concentrations in pernicious anemia. J Clin Investigation 34 948 June 1955 (in Soc Proc)

Therapy with folic acid alone sometimes has a neuropathic effect in pernicious anemia. A study was therefore made of the effect of folic acid on serum vitamin B₁₂ concentrations in the disease.

Twenty seven patients with pernicious anemia who had been receiving 15 mcg of vitamin B₁₂ intramuscularly every four weeks for two or more years were selected. During the first six months 20 were given 30 mcg and 7 were given 20 mcg of vitamin B₁₂ intramuscularly every two weeks. During the next six months all received the same vitamin B₁₂ dosage and in addition took 5 mg of folic acid daily mouth daily. Serum vitamin B₁₂ levels were estimated microbiologically initially.

and after 6 and 12 months using *Euglena gracilis*

Initially 14 of the 27 patients had serum vitamin B₁₂ levels of less than 100 after the first six months only 1 patient had a level of less than 100 and the average was 451. After the second six months of supplemental folic acid the average level was 279. At this time, individual serum vitamin B₁₂ levels in 18 of the 27 patients were at least 15 per cent lower than after the first six months but no neuro pathic effects were observed. Results indicate that injection of 15 mcg of vitamin B₁₂ every four weeks may be insufficient to saturate body vitamin B₁₂ stores. Administration of folic acid may lower the serum level of vitamin B₁₂ possibly by accelerating its utilization.

Boston Mass

7 Das Gupta, C R, Chatterjee, J B, Ghosh, S K. and Banerjee D K. Vitamin B₁₂ concentration of serum in nutritional macrocytic anaemia. Bull Calcutta School Trop Med 3 101, 1955 (abstr Blood 11 688-689 July 1956)

Mean values for total and combined concentrations of vitamin B₁₂ in serum in 6 cases of nutritional macrocytic anaemia estimated microbiologically with *Euglena gracilis* var *bacillaris* as the test organism were respectively 75 (range 20 to 170) and 72 (range 20 to 150) µmcg per cc. These values were significantly lower than the corresponding values in normal Indians. After treatment with specific antimegaloblastic drugs the values of both combined and total vitamin B₁₂ attained normal levels. The findings suggest that in an average case of nutritional macrocytic anaemia as seen in India there is deficiency of vitamin B₁₂.

School of Tropical Medicine
Calcutta India

8 Foy, H, Kondo, A and Manson Bahr, P M C. Penicillin in megaloblastic anaemias of Africans. Effect on serum vitamin B₁₂ levels and absorption of radioactive vitamin B₁₂. Lancet 2 693-699, Oct 1, 1955

Nonpernicious megaloblastic anaemia was studied in African natives. Red cell counts were 600 000 to 2,000 000 per cumm and reticulocyte counts 1 per cent when the patients were admitted. The patients were observed for 3 to 10 days then serum vitamin B₁₂ levels and excretion of radioactive vitamin B₁₂ were studied. The patients were then given 400 000 units of penicillin intramuscularly daily for 6 to 12 days, if there was no response. 80 mcg of vitamin B₁₂ was given intramuscularly or orally. If no response was yet seen folic acid was administered orally. The authors state that this order of giving medication is important since all cases respond to folic acid but not all respond to vitamin B₁₂ or to penicillin.

The patients who responded completely to penicillin or to vitamin B₁₂ had low vitamin B₁₂ serum levels (20 to 100 micromicrograms per cc) before treatment. The authors found that penicillin increased the serum vitamin B₁₂ levels but were uncertain if this increase was due to improved absorption, to reduction of the organisms competing for vitamin B₁₂ or to metabolic changes in the host.

Patients responding to folic acid had normal or high vitamin B₁₂ serum levels before treatment (300 to 1,300 micromicrograms per cc). Patients with serum levels below 700 micromicrograms who eventually improved with folic acid had also shown a slight reticulocytosis after penicillin.

Diets in Africa are generally low in animal protein and rich in carbohydrates but it is not known whether they are deficient in vitamin B₁₂ or folic acid or in both. The diet may encourage growth in the gastrointestinal tract of bacterial competitors for vitamin B₁₂. At a certain time of year the anaemia that responds to penicillin is predominant, while at other times

the type that responds to folic acid pre dominates

*Wellcome Trust Research Laboratories and
Kenya Medical Service
Kenya Colony East Africa*

- 259 Murata K. and Miyamoto T. The determination of vitamin B₁₂ activity in human blood. *J Vitaminol* 1 297 304 Oct 10 1955

Authors' summary. The procedures for releasing and extracting vitamin B₁₂ from proteins in whole blood were investigated by the microbiological assay using *Lactobacillus leichmannii* ATCC 4797

The treatment of the whole blood by heating with potassium cyanide was found to give constant values at each level of the vitamin, and good recoveries of the added vitamin in the assay

The individual values of the vitamin in whole blood estimated by heating with potassium cyanide are in agreement with those obtained by using sodium meta bisulfite and those by papain digestion but not with those by digestion with the trypsin preparation available in this country

The vitamin B₁₂ content of healthy human whole blood was found to range from 0.10 to 0.66 mcg/cc the average value being 0.28 ± 0.05 (U.S.A.) and 0.37 ± 0.07 mcg/cc (Japan)

*Osaka City University
Nishi-ku Osaka Japan*

- 260 Dhopeswarker G. A. Trivedi J. C. Kulkarni M. S. Satoskar R. M. and Lewis R. A. Blood proteins and vitamin B₁₂ in vegetarians. *Brit J Nutrition* 10 105 1956 (abstr. *J Am Dietet A* 32 844-846 Sept 1956)

Plasma protein and blood vitamin B₁₂ were determined in five Indian medical students on a vegetarian diet which included milk and milk products but no other food of animal origin and in five students whose diet included eggs

fish fowl or meat daily. The vegetarians were given 50 mg Aureomycin daily for two months and determinations were repeated after one or two months. The albumin content of the plasma was below normal and the globulin content above normal in the vegetarians who also had a lower blood content of vitamin B₁₂. The administration of Aureomycin to the vegetarians produced a rise in plasma albumin and a fall in all the globulin fractions. Values for blood vitamin B₁₂ increased significantly

- 261 Miller A. Corbus H. F. and Sullivan J. F. The plasma disappearance, excretion and tissue distribution of cobalt⁶⁰ labelled vitamin B₁₂ in normal subjects and patients with chronic myelogenous leukemia. *J Clin Investigation* 36 18-24 Jan 1957

The plasma disappearance, tissue distribution and excretion of a 4 mcg intra venous dose of Co⁶⁰ vitamin B₁₂ were studied in control subjects and in patients with leukemia. The dosage used is about four times the binding capacity of normal serum but is within the total binding capacity of chronic myelogenous leukemia serum.

Plasma radioactivity declined rapidly in the 7 normal subjects, in 1 patient with chronic myelogenous leukemia in remission and in 1 patient with chronic lymphocytic leukemia. The 7 patients with chronic myelogenous leukemia however showed a slow decline in plasma radioactivity while 2 with myeloid metaplasia showed an intermediate plasma disappearance rate. Normal subjects excreted 1 to 4 per cent of the administered dose in the urine within 24 hours compared with 0 to 2 per cent excreted by chronic myelogenous patients. Stool collections from 11 normal subjects showed no radioactivity. In 1 patient with cirrhosis the bile contained 2 per cent of the dose and the ascitic fluid none.

Normal subjects showed an increase

in liver radioactivity throughout the period of observation which was not associated with a comparable fall in plasma radioactivity. Radioactivity was not concentrated in the spleen or white cells of leukemia patients.

The serum vitamin B₁₂ concentration ranged from 364 to 530 micromicrograms per cc in 3 normal subjects and from 2,046 to 9,266 micromicrograms per cc in 4 patients with chronic myelogenous leukemia. It was 704 in a patient with myeloid metaplasia and 2,104 in the patient with cirrhosis. There was no correlation between the leukocyte count and the serum vitamin B₁₂ levels.

The increased vitamin B₁₂ binding power of the plasma of patients with active chronic myelogenous leukemia may aid in differentiating this leukemia from leukemoid states.

*Boston University School of Medicine
Tufts University School of Medicine and
Boston Veterans Administration Hospital
Boston, Mass.*

- 262 Grossowicz, N., Hochman, A., Aro-novitch, J., Izak, G. and Rachmilewitz, M. Malignant growth in the liver and serum vitamin B₁₂ levels, *Lancet* 1:1116-1117, June 1, 1957.

Serum vitamin B₁₂ levels were measured in 35 healthy people, in 18 patients with tumors and metastases of the liver and in 19 with malignant diseases not involving the liver.

Serum values of normals ranged from 200 to 500 micromicrograms per cc (assay of *Esch coli*). The 19 patients without liver metastases had normal serum vitamin B₁₂ levels (100 to 550 micromicrograms per cc). However, 16 of the 18 patients with liver metastases had raised serum vitamin B₁₂ levels of 640 to 20,000 micromicrograms per cc. The highest level occurred in a patient with total gastrectomy, jaundice and extensive liver metastases. One patient with multiple myeloma and extensive amyloidosis

had raised vitamin B₁₂ levels while 2 patients with multiple myeloma not complicated by amyloid had normal levels of vitamin B₁₂. In 1 patient with hepatoma, cirrhosis and a raised serum vitamin B₁₂ level, autopsy revealed extensive infiltration of the liver. In another patient with hepatoma and a normal serum vitamin B₁₂ level, the tumor was limited to a circumscribed area.

A tumor invading the liver by causing necrosis and displacement of liver cells causes the stored vitamin B₁₂ to be released into the blood. Because the process in cancer is prolonged, a persistent rise is to be expected and was in fact found in 3 patients in whom estimations were repeated throughout the illness.

These findings suggest that determination of the serum vitamin B₁₂ may help the diagnosis of metastases to the liver in cases of malignancy. Raised levels should be interpreted as an indication that the liver is involved. Normal levels, however, do not rule out this possibility, because metastasizing tumors not causing liver damage could be present in the liver without raising the serum vitamin B₁₂ level.

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Hadassah Medical School
Jerusalem, Israel*

- 263 Bertcher, R. W., Meyer, L. M. and Cronkite, E. P. Serum Co⁵⁷ vitamin B₁₂ binding capacity in some hematologic disorders. *Clin. Research Proc.* 5:143-144, April 1957.

It has been recently reported that the range of Co⁵⁷ vitamin B₁₂ binding capacity of normal human serum may be estimated by incubating the radioactive vitamin with serum and dialyzing against running tap water for 48 hours. This binding capacity has been studied in various hematologic disorders with the following results:

Ten of 13 cases of chronic myelocytic leukemia showed an elevated binding ca-

capacity which was roughly but not regularly proportional to total leukocyte count. Those cases showing normal binding were under treatment and in remission. One of six cases of chronic lymphocytic leukemia showed moderate and two slight increase of binding capacity. This varied with leukocyte count but was less elevated than in chronic myelocytic leukemia. Increased binding capacity was also found in one of seven adult cases of acute leukemia, two cases of chronic idiopathic leukemia, one of two cases of polycythemia vera and none of three cases of pernicious anemia in relapse.

The findings in chronic myelocytic leukemia and polycythemia vera parallel reports of increased serum content of vitamin B₁₂ in these diseases.

In none of the conditions studied was binding capacity reduced.

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- 264 Bertcher R W and Meyer L M
Co⁵⁷ vitamin B₁₂ binding capacity of
normal human serum Proc Soc Ex
per Biol & Med 94 169 171 Jan
1957

This study presents a method of estimating the Co⁵⁷ vitamin B₁₂ binding capacity of human serum with data on 40 normal subjects. As the amount of Co⁵⁷ vitamin B₁₂ added to the serum was increased the binding capacity of the serum increased. The higher binding capacity shown by this method of measurement compared to that using microbiological assay is not due to ionic cobalt. The discrepancy occurs because the amount of vitamin B₁₂ made available to a microorganism by heating serum does not represent the total vitamin bound to the serum proteins. This vitamin is bound to alpha globulin at usual body levels but at higher concentrations this mechanism may be saturated and nonspecific

binding to other serum protein fractions may take place

5th Nassau Community Hospital
Oceanside, N. Y.

- 265 Sheely L L, Miller O N and Unglaub W G
Vitamin B₁₂ levels in serum and urine following parenteral administration of crystalline vitamin B₁₂ or liver extract Proc Soc Exper Biol & Med 94 629-631 April 1957

Serum and urine levels of vitamin B₁₂ were determined in normal subjects 8 of whom received 50 mcg of crystalline vitamin B₁₂ parenterally and 8 of whom were given an equivalent amount of vitamin B₁₂ in the form of purified liver extract. Blood samples were drawn before injection and 1, 4, 8 and 24 hours after injection. Urine was collected for 24 hours after injection. Total serum vitamin B₁₂ levels, bound vitamin B₁₂ levels and urinary excretion of vitamin B₁₂ were compared in the two groups.

The average maximum serum concentration of total vitamin B₁₂ was 1.8 mcg in the group receiving crystalline vitamin B₁₂ compared to 1.2 mcg in the group receiving liver extract. Concentration of bound vitamin B₁₂ was 0.8 mcg in both groups. Maximum serum concentration of vitamin B₁₂ was reached faster in subjects receiving crystalline vitamin B₁₂. Calculation of the average proportion of bound vitamin to total vitamin over a 24 hour period demonstrated that about 81.5 per cent of vitamin B₁₂ was bound following administration of liver extract compared to 84.9 per cent after injection of crystalline vitamin B₁₂. Urinary excretion of vitamin B₁₂ in the group receiving crystalline vitamin B₁₂ averaged 90 ± 0.75 mcg compared to an average excretion of 60 ± 1.1 mcg in the group receiving liver extract. This was considered significantly lower. It was attributed to the lower proportion of free vitamin B₁₂ present after administration of liver extract.

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New Orleans, La.

time needed for depletion of stores of vitamin B₁₂ and development of macrocytic anemia after total gastrectomy as well as long remissions observed in pernicious anemia following treatment with liver extracts or vitamin B₁₂

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New York N.Y.*

269 Turnbull A Experiences with labelled vitamin B₁₂ Proc. Roy Soc Med. 47 424-426 June 1954

Between 20 and 40 per cent of orally administered radioactive vitamin B₁₂ (0.5 mcg) was recovered from the feces of 10 control subjects compared to an 80 to 93 per cent recovery from 22 patients with pernicious anemia in remission. Since nearly all the pernicious anemia patients were receiving injections of vitamin B₁₂ it was concluded that perhaps they were excreting large amounts of the radio active preparation because their tissues were already saturated with the vitamin. Radioactive vitamin B₁₂ was therefore given with intrinsic factor to 7 patients with pernicious anemia. In these 7 patients recovery of radioactivity was much reduced and with the more potent intrinsic factor preparations the recoveries approached those found in the control patients. It was concluded that the difference between the findings in the control subjects and the patients with pernicious anemia was due to the failure of the latter to secrete intrinsic factor.

The same lack of intrinsic factor apparently occurs after total gastrectomy indicating that in man the stomach is the only gastrointestinal source of intrinsic factor.

Normal recoveries were made in 3 patients with steatorrhea who apparently had normal secretion of intrinsic factor and absorption of vitamin B₁₂. In 2 patients however the recoveries were greater than normal but were not reduced by the addition of intrinsic factor. Probably the defect in absorption in these patients was not due to lack of intrinsic factor. In

1 patient the recovery was similar to that from patients with pernicious anemia and it was greatly reduced when intrinsic factor was also given.

In 2 patients with megaloblastic anemia of pregnancy (both receiving folic acid) the recoveries of radioactivity were within normal limits. Therapy with folic acid may have caused resumption of the secretion of intrinsic factor in these patients or its return to normal levels though it does not have this effect in Addisonian pernicious anemia.

The drawbacks of this technique of estimating vitamin B₁₂ excretion and absorption are pointed out but it is stated that it nevertheless appears to provide a useful indirect measure of the secretion of intrinsic factor.

*The Radcliffe Infirmary
Oxford England*

270 Krevans J R Conley C L and Sachs M Influence of certain diseases on the absorption of vitamin B₁₂ from the gastrointestinal tract J Clin Investigation 33 949-950 June 1954 (in Soc Proc)

In 16 normal individuals administration of 0.1 to 0.5 mcg of Co⁵⁷ vitamin B₁₂ was followed by recovery of 10 to 47 per cent of the radioactivity in the stool. Within this range there was no relationship between the amount of vitamin B₁₂ administered and the per cent recovered. With a dose of 1.0 mcg as much as 60 per cent of the radioactivity was recovered and with 5.5 mcg more than 70 per cent. In 23 studies on patients with achlorhydria liver disease or anemia unrelated to vitamin B₁₂ deficiency administration of 0.1 to 0.5 mcg of labelled vitamin B₁₂ was followed by recovery of 10 to 45 per cent. One 21 year-old man with hepatitis and normal gastric acidity excreted 67 per cent.

In 8 patients with pernicious anemia recovery of radioactivity was more than 60 per cent after a dose of 0.5 mcg vitamin B₁₂. One patient who appeared to

have typical pernicious anemia excreted less than 35 per cent and the Schilling test indicated apparently normal vitamin B₁₂ absorption. One of 3 patients with total gastrectomy and 2 of 3 with sprue showed impaired absorption. One patient with megaloblastic anemia subacute combined degeneration normal gastric acidity and multiple diverticula of the jejunum demonstrated markedly impaired absorption which became normal during Aureomycin administration. Terramycin failed to produce normal absorption in a patient with pernicious anemia.

Baltimore Md

- 271 Glass G H J, Boyd, L J and Stephanson L. Intestinal absorption of vitamin B₁₂ in man. *Science* 120: 74-75 July 9, 1954 (in Correspondence)

For the purpose of studying the absorption of vitamin B₁₂ following parenteral and oral administration the authors used surface scintillation measurements for the uptake of radioactive vitamin B₁₂ by the liver. These studies demonstrated an inverse relationship between the radioactivity counts over the liver and the amounts of crystalline vitamin B₁₂ added to the radioactive vitamin B₁₂ ingested.

The intestinal absorption of vitamin B₁₂ under normal conditions is apparently controlled by a partial mucosal block similar to that regulating intestinal iron absorption. This partial mucosal block changes to a complete or almost complete block in sprue and in pernicious anemia. This is indicated by decreased or absent hepatic uptake of orally administered vitamin B₁₂ in these diseases. In sprue the block cannot be corrected by the addition of intrinsic factor because the absorption mechanism of the intestinal wall is inherently defective. In pernicious anemia however the block can be converted into a partial block by administration of normal human gastric juice or intrinsic factor concentrate.

Merely increasing greatly the intake of vitamin B₁₂ overcomes the block and results in some absorption. However the principle of regressing efficiency of vitamin B₁₂ absorption in the intestine with increase of the intake still applies.

New York Medical College
Flower and Fifth Avenue Hospitals
New York N Y

- 272 Halsted, J A, Gasster M and Drenick E J. Absorption of radioactive vitamin B₁₂ after total gastrectomy. *New England J Med.* 251: 161-168, July 29, 1954

The absorption of orally administered, radioactive vitamin B₁₂ was studied by using a scintillation counter to determine its fecal excretion. In 11 normal persons excretion on the average was 33 per cent of a 0.5 mcg dose. In 3 patients with idiopathic achlorhydria the average was 34 per cent. In 7 patients with pernicious anemia the average excretion was 93 per cent in 10 tests. When intrinsic factor was administered with the test dose in 4 of these patients fecal excretion was decreased to an average of 38 per cent. The average excretion in 11 totally gastrectomized patients was 87 per cent in 16 tests. When a source of intrinsic factor was administered with the test dose an average of only 20 per cent was excreted in 14 tests.

None of the 11 gastrectomized patients had macrocytic anemia, but 9 had received therapy to prevent it. However 10 were significantly underweight, 7 had normocytic anemia and 10 had steatorrhea.

Reasons why macrocytic anemia rarely follows total gastrectomy are: (1) Few patients survive long enough for anemia to develop. (2) Liver stores of vitamin B₁₂ may be sufficient for several years. (3) In many operations considered total gastrectomies part of the cardia which secretes intrinsic factor remains and (4) most patients receive prophylactic anti-anemic therapy.

Los Angeles Calif

- 273 Bradley J E, Smith E L, Baker S J and Mollin D L The use of the radioactive isotope of cobalt Co^{58} for the preparation of labelled vitamin B_{12} *Lancet* 2 476-477, Sept 4 1954

Co^{58} has a half life of only 72 days compared to a half life of 5 years for Co^{60} . It is therefore suggested for reasons of safety that Co^{58} instead of Co^{60} be used in clinical excretion studies of vitamin B_{12} .

Postgraduate Medical School of London
London, England

Glaxo Labo stories
Glenford Middlesex, England

- Franz, W and Pendl I Influence of bacterial flora of stomach on absorption of orally administered vitamin B_{12} *Klin. Wchnschr* 32 1092-1096 Dec. 1 1954 (abstr. *JAMA* 157 57 March 12, 1955)

The gastric juice of 3 patients with severe untreated pernicious anemia contained no intrinsic factor or bound vitamin B_{12} . The authors were at first unable to culture colon bacilli from the gastric juice of these patients. They all had not only a moderate increase in reticulocytes but also changes in the bone marrow suggesting the onset of a spontaneous remission. Two of the patients were given small doses (5, 10 and 30 mcg) of vitamin B_{12} orally. The condition of one did not improve and it was found that her gastric juice contained paracolonic bacilli which completely consumed or destroyed vitamin B_{12} (added *in vitro*). In the second patient complete remission of the pernicious anemia resulted but only after treatment for 98 days. This patient had paracolonic bacilli in his gastric juice only for a short time and *in vitro* tests revealed only 80 per cent of the added vitamin B_{12} . Colon bacilli found in the gastric juice of the third (untreated) patient destroyed 50 per cent of added vitamin B_{12} . More recently the authors have been able to

alleviate pernicious anemia in another patient by small oral doses of vitamin B_{12} . This patient had no colon bacilli in his gastric juice.

The authors believe that vitamin B_{12} taken in food or in small oral doses can be absorbed even if intrinsic factor is absent provided the stomach does not contain colon bacilli that consume or destroy the vitamin.

Berlin, Germany

- 275 Glass G B J, Pack, G T and Mersheimer, W L Uptake of radioactive vitamin B_{12} by the liver in patients with total and subtotal gastrectomy *Gastroenterology* 29 666-683 Oct 1955

Intestinal absorption of vitamin B_{12} was studied by direct measurement of the hepatic uptake of Co^{58} vitamin B_{12} . In normal subjects ingestion of radioactive vitamin B_{12} results in accumulation of radioactivity in the liver. In patients with pernicious anemia radioactivity accumulates in the liver only if intrinsic factor is given with the oral vitamin B_{12} . The hepatic uptake of vitamin B_{12} by 7 of 8 subtotal gastrectomized patients was at the lower level of the normal range and above the normal mean in 1. When intrinsic factor was given with oral vitamin B_{12} to 4 of these patients hepatic uptake doubled or tripled in 3 but changed little in the patients whose uptake of vitamin alone was above the normal mean.

The 8 totally gastrectomized patients showed hepatic uptake of oral vitamin B_{12} only when intrinsic factor was given.

Presumably the prolonged hepatic storage of vitamin B_{12} explains the delayed appearance of anemia after total gastrectomy. When radioactive vitamin B_{12} was given intramuscularly to normal subjects or to patients with pernicious anemia or sprue 65 to 86 per cent of the peak radioactivity persisted in the liver two or three months after injection. The long interval between total gastrectomy

several years (2) gastrectomy may have been incomplete or (3) the patient may have been given liver extract or vitamin B₁₂ prophylactically. Evidence indicates that every patient who does survive long enough without prophylactic therapy will develop megaloblastic anemia indistinguishable from Addisonian pernicious anemia. A monthly injection of 30 mcg of vitamin B₁₂ to prevent anemia is recommended.

The 3 patients with megaloblastic anemia showed high fecal excretion of the test dose of vitamin B₁₂; this did not change with administration of intrinsic factor. Chlorotetracycline or tetracycline therapy however resulted in a significant decrease in vitamin B₁₂ excretion (increased absorption). Bacterial competition for vitamin B₁₂ may have been the mechanism involved in producing the anemia. An injection of 30 mcg of vitamin B₁₂ once a month in such patients controls the deficiency causing the anemia.

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Los Angeles Calif*

- 279 Schilling R. F. Clatanoff, D. V. and Korst D. R. Intrinsic factor studies. III. Further observations utilizing the urinary radioactivity test in subjects with achlorhydria, pernicious anemia or a total gastrectomy. *J Lab & Clin Med* 45:926-934 June 1955.

To test absorption of vitamin B₁₂, the radioactive vitamin was administered by mouth to 97 subjects and followed by an intramuscular injection of nonradioactive vitamin B₁₂; measurements of urinary radioactivity were then made.

The 111 control subjects excreted from 7 to 22 per cent of the radioactivity in 24 hours (average 14.2 per cent). In 31 achlorhydric subjects with no history of pernicious anemia excretion ranged from 2.2 to 29 per cent (average 11.6 per cent). The 35 patients with pernicious anemia ex-

creted 0 to 2.3 per cent when the vitamin was given without intrinsic factor. When the same dose of the radioactive vitamin was given with intrinsic factor, these patients excreted from 3.1 to 30 per cent (average 9.8 per cent). The 13 totally gastrectomized subjects were entirely similar to pernicious anemia patients in their inability to absorb vitamin B₁₂.

The author states that the urinary radioactivity test is useful in diagnosis of pernicious anemia and in estimating activity of intrinsic factor preparations.

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Madison Wis*

- 280 Meyer L. M. Berlin N. I. Jimenez Casado M. and Arkum S. N. Vitamin B₁₂ distribution determined by surface body counting following parenteral administration of Co⁵⁷ vitamin B₁₂. *Proc Soc Exper Biol & Med* 91:129-131 Jan 1956.

Co⁵⁷ vitamin B₁₂ 1 mcg was given intramuscularly to 2 normal subjects and to 12 patients with various diseases (arteriosclerosis, rheumatoid arthritis, cirrhosis, pernicious anemia, Gaucher's disease, lymphosarcoma or polycythemia). Radioactivity was measured over several body sites beginning 30 minutes after the labeled vitamin B₁₂ was injected.

No significant radioactivity following Co⁵⁷ vitamin B₁₂ injection was found over the precordium, sacrum or thighs. Radioactivity was always detectable over the liver, spleen and left kidney within 30 minutes after injection. Levels were highest over the liver. Radioactivity disappeared from the site of injection within three to four hours.

Six subjects were given Co⁵⁷ Cl₂ (radioactive cobalt chloride) intramuscularly. The pattern of absorption from the injection site and the distribution over the liver, spleen and kidney were entirely different from those following Co⁵⁷ vitamin B₁₂ administration. Thus, in studies with Co⁵⁷ vitamin B₁₂, it is probable that

the radioactivity measured represents vitamin B₁₂ with Co⁶⁰ still attached

*Goldwater Memorial Hospital
New York N Y*

*University of California
Berkeley Calif*

- 31 Unglaub, W G , Miller, O N and Goldsmith, G A Saturation studies with vitamin B₁₂ in human subjects Federation Proc 15 374 March 1956

Normal subjects patients with macrocytic anemia and a group of patients with miscellaneous diseases were given vitamin B₁₂ 50 mcg intramuscularly daily for 10 days followed by 1000 mcg daily for an additional 10 days After a 3 day interval, a final test dose of 50 mcg was administered Urinary excretion of the vitamin and concentrations of free and bound vitamin B₁₂ in serum were measured at suitable intervals There was a significant increase in the concentration of free and bound vitamin B₁₂ during the period of saturation in all subjects which in most instances was still present 3 days after the saturation period In all patients with pernicious anemia tested thus far and in some patients with other types of macrocytic anemia the maximum level of bound vitamin B₁₂ was significantly less than that attained in normal subjects or in patients with miscellaneous diseases During the period of administration of 50 mcg of the vitamin urinary excretion increased progressively for the first 5 days after which it was maintained at approximately a constant level in most subjects Excretion varied widely among individuals and among groups at all levels of saturation No correlation was observed between urinary excretion of the vitamin and concentration of free bound or total vitamin B₁₂ in the serum

*Tulane University
New Orleans La*

- 32 Best W R Landmann W A and Limarzi, L R Time pattern of vitamin B₁₂ Co⁶⁰ urinary excretion in man

after oral administration and parenteral flushing' Blood 11 352 356 April 1956

Serial urine collections in 8 patients with pernicious anemia given 2 mcg Co⁶⁰ vitamin B₁₂ orally followed in two hours by 1 000 mcg nonradioactive parenteral vitamin B₁₂ showed little radioactivity When the same doses were repeated with a potent oral dose of intrinsic factor concentrate peak excretion of radioactivity usually occurred between 8 and 12 hours after ingestion of radioactive vitamin B₁₂, sometimes even later The excretion rate dropped relatively low after 12 to 24 hours in most patients Similar curves were seen in controls given no intrinsic factor Secondary peaks occasionally occurred

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College of Medicine and
Armour Laboratories
Chicago Ill*

- 283 Miller A , Corbus, H F and Sullivan, J F The plasma disappearance excretion and tissue distribution of intravenous Cobalt⁶⁰ vitamin B₁₂ in normal subjects with chronic myelogenous leukemia, J Clin Investigation 35 724, June 1956 (in Soc Proc)

After 4 mcg of Co⁶⁰ vitamin B₁₂ was injected intravenously into normal subjects and leukemic patients its disappearance from the plasma tissue distribution and urinary and fecal excretion were determined A rapid decline in plasma radioactivity occurred in normal subjects 7 to 12 per cent of the dose remained at 2 hours and 4 to 10 per cent at 24 hours Urine and stools contained negligible amounts of radioactivity In 7 out of 8 patients with myelogenous leukemia plasma radioactivity disappeared more slowly 50 to 63 per cent of the dose remained at two hours and 38 to 44 per cent at 24 hours After 24 hours the disappearance rate decreased (half time of five days) One patient in remission from chronic myelogenous leukemia 2 with

myeloid metaplasia and I with chronic lymphocytic leukemia had normal rates of disappearance

External monitoring showed an increase in liver radioactivity throughout the period of observation (7 to 22 days) not associated with a comparable fall in plasma radioactivity. The leukemic patients showed a smaller rise in hepatic radioactivity than did the normals. In 2 myelogenous leukemic patients the liver at autopsy contained 39 to 42 per cent of the administered dose, the spleen 8 to 11 per cent and the other viscera less than 1 per cent. Red and white cells from both groups of patients contained no radioactivity.

The increased *in vivo* plasma binding of vitamin B₁₂ in myelogenous leukemia without preferential concentration in leukemic tissue suggests that the turnover of vitamin B₁₂ in leukemic tissue is not increased. The decreased plasma disappearance may prove to be of diagnostic value in differentiating chronic myelogenous leukemia from leukemoid states.

Bo Ion Afari

- 284 Diet and anemia J A M A 161 474
June 2 1956 (in Foreign Letters
United Kingdom)

At a symposium of the Nutrition Society Dr F Wokes reported on a sect of 700 vegetarians who eat no animal food whatsoever. Their protein intake is only about 10 to 12 Gm daily. Although their hematograms are not like those of pernicious anemia these patients do suffer from subacute combined degeneration of the spinal cord which caused 2 deaths. This is attributed to insufficient intake of vitamin B₁₂ combined with a high intake of folic acid.

Dr J R Evans of London described absorption studies made with radioactive vitamin B₁₂. In patients with pernicious anemia only 10 per cent of a dose of vitamin B₁₂ is absorbed compared to a normal absorption of 50 to 75 per cent. Ab-

sorption is increased if intrinsic factor is administered. Patients who have had a total gastrectomy show diminished absorption because the source of intrinsic factor has been removed. With partial gastrectomy however this area is retained. Patients with idiopathic steatorrhea and intestinal infections also showed diminished absorption of vitamin B₁₂. It is doubtful if the vitamin B₁₂ formed in the intestines by bacterial action is available because if the intestinal wall is sterilized the amount of the vitamin absorbed is unchanged.

- 285 Doscherholmen A and Hagen P S
Radioactive vitamin B₁₂ absorption studies: results of direct measurement of radioactivity in the blood J Clin Investigation 35 699 June 1956 (in Soc Proc)

The absorption of vitamin B₁₂ was determined by measurement of radioactivity of blood samples collected after the oral administration of a test dose of radioactive vitamin B₁₂. Test doses consisted of 0.46 mcg and 0.92 mcg of vitamin B₁₂ containing 0.5 microcurie and 1.0 microcurie of Co⁶⁰ respectively. Peak radioactivity in the blood samples did not appear until 8 to 12 hours after the test dose was given.

Seventeen of 20 non pernicious anemia patients were given the 0.46 mcg dose. In 6 tests whole blood net counts ranged from 18 to 41 p.c. minute while in 14 instances plasma counts ranged from 19 to 54. In 3 given the larger test dose whole blood net counts ranged from 33 to 55 while plasma counts were 56 to 108. In 4 patients with pernicious anemia given the 0.46 mcg 0.5 microcurie dose the blood and plasma counts were not significant ranging from 2 to 6 per minute. In one pernicious anemia patient given the dose containing 1.0 microcurie of Co⁶⁰ net counts of 10 and 11 per minute were obtained in the whole blood and plasma respectively. However with po-

tent intrinsic factor concentrate the blood or plasma radioactivity was within the range found in the non pernicious anemia patients

Minneapolis Minn

- 286 Pollycove, M and Apt L Absorption, elimination and excretion of orally administered vitamin B₁₂ in normal subjects and in patients with pernicious anemia, *New England J Med* 255 207-212, Aug 2, 1956

Knowledge of the intestinal absorption of vitamin B₁₂ is valuable in the diagnosis of addisonian pernicious anemia in patients with achlorhydria who are receiving maintenance therapy with liver or vitamin B₁₂. Such absorption may be tested by measuring the hepatic uptake of orally administered Co⁵⁷ vitamin B₁₂, or its excretion in urine or feces. All three methods were used in 3 pernicious anemia patients and in 3 normal subjects with fairly consistent results. The advantages of the three methods are discussed. The urinary excretion test is most rapid but does not directly measure the amount of vitamin absorbed. The liver scintillation count is the simplest, easiest, and most accurate but requires at least seven days for completion. The fecal excretion method takes at least 10 days but provides a direct measurement of the amount of vitamin B₁₂ excreted.

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- 287 Bunge, M B, Schloesser L L and Schulling R F Intrinsic factor studies. IV Selective absorption and binding of cyanocobalamin by gastric juice in the presence of excess pseudovitamin B₁₂ or 5,6-dimethylbenzimidazole. *J Lab & Clin Med* 48 735 744, Nov 1956

The selective absorption and binding of vitamin B₁₂ by gastric juice and serum

in the presence of pseudovitamin B₁₂ or 5,6-dimethylbenzimidazole (a moiety of the cyanocobalamin molecule) were studied *in vitro* and also in 6 patients who lacked intrinsic factor (pernicious anemia 4, subtotal gastrectomy 2).

Each patient received 1 000 mcg of crystalline vitamin B₁₂ subcutaneously two hours after orally administered doses of normal human gastric juice and 1 mcg Co⁵⁷ vitamin B₁₂ plus 50 mcg vitamin B₁₂, 50 mcg pseudovitamin B₁₂ or 5 mcg 5,6-dimethylbenzimidazole. Neither pseudovitamin B₁₂ nor 5,6-dimethylbenzimidazole had any effect or showed competition with radioactive cyanocobalamin for mechanisms affecting vitamin B₁₂ absorption.

To test flushing action, 1 000 mcg doses of vitamin B₁₂ and pseudovitamin B₁₂ were administered subcutaneously two hours after administration of 1 mcg of oral Co⁵⁷ vitamin B₁₂ to 2 patients with pernicious anemia and to 4 control subjects. The urine radioactivity was markedly less when pseudovitamin B₁₂ was used although it had some flushing action.

In vivo binding was studied in 2 control subjects. Similar amounts of vitamin B₁₂ or pseudovitamin B₁₂ were excreted in the urine 24 hours after intramuscular injection of 122 mcg. Administering excess vitamin B₁₂, pseudovitamin B₁₂ or 5,6-dimethylbenzimidazole showed that the *in vitro* binding of cyanocobalamin by gastric juice was a selective process with a preference for vitamin B₁₂ over pseudovitamin B₁₂ and 5,6-dimethylbenzimidazole.

The binding capacity of serum was not entirely selective. Both vitamin B₁₂ and pseudovitamin B₁₂ reduced binding of Co⁵⁷ vitamin B₁₂ by serum. 5,6-dimethylbenzimidazole however did not have this effect.

The authors conclude: The data are in accord with the hypothesis that binding of cyanocobalamin is necessary for intrinsic factor activity but binding alone

is no criterion for the presence of such activity

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Madison, W. Is

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Rahway, N. J.

- 288 Rath C. E. McCurdy P. R. and
Duffy B. J. Jr. Effect of renal dis-
ease on the Schilling test. *New Eng-
land J. Med.* 256:111-114 Jan 17
1957

The widespread use of panhematinics containing folic acid has made the diagnosis of pernicious anemia difficult. The Schilling test utilizing Co^{57} vitamin B_{12} which has become a valuable adjunct in diagnosis, may be limited in renal disease. The results of this test were studied in 33 control subjects in 29 patients with pernicious anemia and in 16 with severe renal disease. Each subject (fasting) was given 1 000 mcg of nonradioactive vitamin B_{12} intravenously 30 minutes after the ingestion of 0.1 microcurie of Co^{57} vitamin B_{12} made up to a total of 0.5 mcg. A few patients received daily intramuscular flushing doses of 1 000 mcg of nonradioactive vitamin B_{12} .

The 24-hour urinary excretion in the 29 patients with Addison's pernicious anemia was less than 6.9 per cent of the oral dose. The 33 normal subjects excreted 8.4 per cent or more; the majority over 10 per cent. Of the 16 patients with renal disease 13 excreted 5.7 per cent or less of the oral dose. There was no correlation between urinary volume, blood urea nitrogen and the urinary excretion of the radioactive vitamin. Intrinsic factor administered to 2 patients did not increase the excretion of the vitamin. This suggests that the defect is not an effect of uremia on intrinsic factor formation. However, there was a considerable excretion of radioactive vitamin B_{12} after the first 24 hours in the 7 patients with renal disease in contrast to the 3 patients with pernicious anemia. These observations suggest that the low rates of excretion in

some patients with renal disease are due to a delay in excretion resulting from impaired glomerular filtration in the presence of relatively intact tubular reabsorption. This delayed excretion might be a useful aid in the differential diagnosis of pernicious anemia and renal disease in which routine urinalysis and clinical evaluation disclose little information.

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School of Medicine and Hospital
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- 289 Berlyne G. M. Liversedge, L. A.
and Emery E. W. Radioactive vitamin B_{12} in the diagnosis of neurological disorders. *Lancet* 1:294-296
Feb 9 1957

The Schilling test for vitamin B_{12} absorption was used diagnostically in 25 patients with neurologic disorders. The patients were given 0.5 microcurie of Co^{57} vitamin B_{12} to which nonradioactive vitamin B_{12} was added to make a total of 3 mcg of vitamin B_{12} per cc. This was given by mouth in 100 cc of water. Two hours later 1 000 mcg of ordinary vitamin B_{12} was given intramuscularly to ensure high serum levels and maximum excretion. Urine excreted during the 24 hours following ingestion of Co^{57} vitamin B_{12} was assayed for radioactivity.

The test was first done on 5 normal subjects. The excretion was 4.0 to 7.1 per cent, a range lower than the 7 to 22 per cent reported by Schilling. The lower values may have been due to age, since none of the 5 subjects were under 45.

In 10 patients with subacute combined degeneration the excretion range was 0.3 to 1.0 per cent. When the test was repeated in 4 patients with the addition of 100 mg of intrinsic factor to the Co^{57} vitamin B_{12} , the excretion rose to 4.1 to 6.0 per cent.

In 15 patients with myeloneuropathies this test made it possible to rule out subacute combined degeneration, since excretion of vitamin B_{12} was normal.

tent intrinsic factor concentrate the blood or plasma radioactivity was within the range found in the non pernicious anemia patients

Minneapolis Minn

- 286 Pollycove, M and Apt, L Absorption, elimination and excretion of orally administered vitamin B₁₂ in normal subjects and in patients with pernicious anemia, *New England J Med* 255 207-212 Aug 2, 1956

Knowledge of the intestinal absorption of vitamin B₁₂ is valuable in the diagnosis of Addisonian pernicious anemia in patients with achlorhydria who are receiving maintenance therapy with liver or vitamin B₁₂. Such absorption may be tested by measuring the hepatic uptake of orally administered Co⁵⁷ vitamin B₁₂ or its excretion in urine or feces. All three methods were used in 3 pernicious anemia patients and in 3 normal subjects with fairly consistent results. The advantages of the three methods are discussed. The urinary excretion test is most rapid but does not directly measure the amount of vitamin absorbed. The liver scintillation count is the simplest, easiest and most accurate but requires at least seven days for completion. The fecal excretion method takes at least 10 days but provides a direct measurement of the amount of vitamin B₁₂ excreted.

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- 287 Bunge, M B, Schloesser L L and Schilling, R F Intrinsic factor studies. IV Selective absorption and binding of cyanocobalamin by gastric juice in the presence of excess pseudovitamin B₁₂ or 5,6-dimethylbenzimidazole. *J Lab & Clin Med* 48 735-744, Nov 1956

The selective absorption and binding of vitamin B₁₂ by gastric juice and serum

in the presence of pseudovitamin B₁₂ or 5,6-dimethylbenzimidazole (a moiety of the cyanocobalamin molecule) were studied *in vitro* and also in 6 patients who lacked intrinsic factor (pernicious anemia 4 subtotal gastrectomy, 2)

Each patient received 1,000 mcg of crystalline vitamin B₁₂ subcutaneously two hours after orally administered doses of normal human gastric juice and 1 mcg Co⁵⁷ vitamin B₁₂ plus 50 mcg vitamin B₁₂, 50 mcg pseudovitamin B₁₂ or 5 mcg 5,6-dimethylbenzimidazole. Neither pseudovitamin B₁₂ nor 5,6-dimethylbenzimidazole had any effect or showed competition with radioactive cyanocobalamin for mechanisms affecting vitamin B₁₂ absorption.

To test flushing action 1,000 mcg doses of vitamin B₁₂ and pseudovitamin B₁₂ were administered subcutaneously two hours after administration of 1 mcg of oral Co⁵⁷ vitamin B₁₂ to 2 patients with pernicious anemia and to 4 control subjects. The urine radioactivity was markedly less when pseudovitamin B₁₂ was used, although it had some flushing action.

In vivo binding was studied in 2 control subjects. Similar amounts of vitamin B₁₂ or pseudovitamin B₁₂ were excreted in the urine 24 hours after intramuscular injection of 122 mcg. Administering excess vitamin B₁₂, pseudovitamin B₁₂ or 5,6-dimethylbenzimidazole showed that the *in vitro* binding of cyanocobalamin by gastric juice was a selective process with a preference for vitamin B₁₂ over pseudovitamin B₁₂ and 5,6-dimethylbenzimidazole.

The binding capacity of serum was not entirely selective. Both vitamin B₁₂ and pseudovitamin B₁₂ reduced binding of Co⁵⁷ vitamin B₁₂ by serum. 5,6-dimethylbenzimidazole, however, did not have this effect.

The authors conclude: "The data are in accord with the hypothesis that binding of cyanocobalamin is necessary for intrinsic factor activity but binding alone

tients with renal insufficiency emphasizing the importance of collecting the urine beyond 24 hours. Further evidence of delayed excretion was seen when 1 control and 1 patient with renal insufficiency were given 1 000 mcg of labeled vitamin B₁₂ intravenously. In the normal subject 96 per cent of the dose had left the plasma in four hours and 86 per cent was excreted in the urine in 24 hours compared to 78 per cent and 47 per cent in the patient with renal insufficiency.

*Boston Univ. School of Medicine and
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Boston, Mass.*

- 292 Goldberg S R, Trivedi B K, and Oliner L. Radioactive vitamin B₁₂ excretion test and the measurement of absorbed plasma radioactivity. *J Lab & Clin Med* 49:583-589 April 1957.

Vitamin B₁₂ activity in urine and plasma were compared in normal subjects and in patients with pernicious anemia. The urinary test was done on 19 normal and 7 pernicious anemia patients and the plasma studies on 16 normal and 5 pernicious anemia patients. The urinary excretion (modified Schilling test) was measured in the 24-hour urine collected after the patient received 0.46 mcg of Co⁶⁰ vitamin B₁₂ orally followed by one hour by a 100 mcg flushing dose of non-radioactive vitamin B₁₂ given intramuscularly. Plasma radioactivity was measured after 1 mcg or more of either Co⁶⁰ or Co⁵⁷ vitamin B₁₂ was given orally with or without a flushing injection. Smaller doses did not give detectable plasma levels.

The 24-hour urinary excretion of Co⁶⁰ vitamin B₁₂ in healthy subjects ranged from 6.2 to 33.4 per cent (mean 17.9 per cent) of the dose while pernicious anemia patients excreted from 0.20 to 3.74 per cent. Of 5 patients with renal disease 3 had excretion within the normal range but 2 with low urine volumes fell within the pernicious anemia range.

Peak plasma radioactivity following 2 mcg or less of vitamin B₁₂ occurred 10 hours after ingestion then declined but was still significant at 24 hours. The range was 0.23 to 1.71 per cent (mean 0.8 per cent) of dose per liter of plasma for normal subjects and a maximum of 0.12 per cent per liter of plasma in patients with pernicious anemia. Previous studies showed that following potent intrinsic factor preparations absorption curves of pernicious anemia patients were similar to those of normal subjects.

Maximum plasma radioactivity following 4 mcg doses of vitamin B₁₂ in the 3 normal subjects given this dose was about half the mean value of 0.8 per cent found at 10 hours after the smaller dose. This apparently bore out previous reports that gastrointestinal absorption of vitamin B₁₂ decreased if the amount given exceeds 1.5 to 2.0 mcg. One of the 2 pernicious anemia patients given 4 mcg had plasma radioactivity levels within the normal range. This is said to imply that with larger amounts of vitamin B₁₂ there may be a process other than active absorption possibly simple diffusion.

The authors state that the plasma radioactivity method is advantageous since it does not require the patient to collect a specimen.

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Chicago, Ill.*

- 293 Doscherholmen A and Hagen P S. Radioactive vitamin B₁₂ absorption studies: results of direct measurement of radioactivity in the blood. *Blood* 12:336-346 April 1957.

A method of studying the absorption of oral vitamin B₁₂ by direct measurement of radioactivity of the blood was tested in 36 control subjects and in 9 patients with pernicious anemia. Oral doses of 0.46 or 0.92 mcg (0.5 or 1 microcurie) of Co⁶⁰ vitamin B₁₂ were given in the fasting state and the radioactivity of the blood or plasma was measured at inter-

A patient with subacute combined degeneration and diabetes excreted only 0.3 per cent of the ingested radioactive vitamin B₁₂. When the test was repeated with the addition of 100 mg of intrinsic factor excretion rose to 4.1 per cent. With vitamin B₁₂ therapy, neurologic symptoms cleared in three months.

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Manchester, England*

- 290 Citrin Y, DeRosa, C and Halsted, J A. Locus of absorption of vitamin B₁₂. *Federation Proc* 16:353, March 1957

In subjects with and without pernicious anemia physiologic doses (0.5 µg) Co⁶⁰B₁₂ was delivered by intestinal intubation to various levels of the intestinal tract. Using 2 flushing doses absorption was assessed by the urinary excretion test, urine being collected for 48 hours. Good reproducibility in the same individual was demonstrated. When Co⁶⁰B₁₂ was delivered to the duodenum of 5 normal subjects from 15.6-39.5% was recovered. In 4 recovery following duodenal instillation was significantly greater than after oral administration. After jejunal instillation 12.3, 22.3 and 22.8% recovery, respectively occurred in 3 subjects. In 6 subjects the test dose delivered to the lower ileum resulted in urinary recoveries of 0, 0, 4.7, 16.5, 18.4 and 23.2% respectively. In 4 patients with pernicious anemia the test dose to which was added a potent intrinsic factor preparation was delivered to the lower ileum. Significant absorption occurred in all. Without added intrinsic factor no absorption was demonstrable. Fourteen test doses alone with intrinsic factor or after Neomycin sterilization instilled into the sigmoid of normal subjects and 1 dose with intrinsic factor instilled into the transverse colon resulted in no absorption. These results suggest that the lower small intestine but not the large bowel, is capable of absorption of B₁₂ in

physiologic amounts and that absorption from the ileum requires the presence of intrinsic factor.

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Syracuse, N. Y.*

- 291 Miller, A, Corbus H F and Sullivan, J F. A modified urinary excretion test for measuring oral cobalt⁶⁰ labeled vitamin B₁₂ absorption and its application in certain disease states. *Blood* 12:347-354, April 1957

Factors influencing the results of the urinary excretion test for oral vitamin B₁₂ include the time of the first flushing dose, the effect of the second flushing dose, and the size of the oral dose. A modified test is presented in which an oral dose of 0.48 mcg of Co⁶⁰ vitamin B₁₂ is given to the fasting patient followed by two flushing doses of 1 mg of vitamin B₁₂ given intramuscularly 3 and 24 hours later. Urine is collected for two 24-hour periods and the radioactivity is measured.

Results of the Modified Test

Disease	Patients	Urinary excretion (% of dose)
Controls	18	21 to 48%
Pernicious Anemia	9	1 to 7%
Combined System Disease without anemia, receiving vitamin B	3	2 to 15%
Bacterial Infection		
Acute	3	1 to 9%
Convalescent	5	18 to 47%
Renal Disease	3	27 to 51%
Terminal	1	0 to 3%
Miscellaneous Diseases	16	17 to 50%

The modified test produces counting rates in normals which are far greater than those in patients with pernicious anemia thus increasing diagnostic accuracy. In other disease states only patients with sprue and a patient with exfoliative dermatitis receiving cortisone had values clearly below the normal range.

Results in patients with acute infection and severe uremia appear invalid. A delay in urinary excretion occurred in pa-

termine the vitamin B₁₂ activity in the urine after nasal application

Normal subjects and pernicious anemia patients excrete vitamin B₁₂ in the urine in similar quantities following parenteral injection. The urinary vitamin B₁₂ activity following intranasal instillation of the vitamin suggests similar patterns but smaller quantities are excreted than after parenteral administration of equal amounts. A pernicious anemia patient in relapse showed a reticulocytosis of 44.3 per cent when 150 mcg. of crystalline vitamin B₁₂ was applied directly to the mucous membrane of the nasal turbinate bone. A single attempt to demonstrate intranasal binding of vitamin B₁₂ with nasal mucus was unsuccessful. Crystalline vitamin B₁₂ in saline solution and as crystals is rapidly absorbed and excreted by the kidneys when applied to the nasal mucosa.

Henry Ford Hospital
Detroit, Mich.

- 296 Monto R. W. and Howell J. T. Urinary vitamin B₁₂ activity following intranasal administration. *J. Lab. & Clin. Med.* 45: 474-477, March 1955.

On separate days nasal instillations of 200, 100 and 50 mcg. of crystalline vitamin B₁₂ in 0.5 cc. saline were given to 2 normal subjects. In addition 2 patients with pernicious anemia in relapse received under direct observation, upon the nasal mucosa 200 mcg. and 150 mcg. respectively of vitamin B₁₂ crystals. Urine specimens were collected for a two-hour period before administration and at two, five, and eight hours afterward. Assays for vitamin B₁₂ activity utilized *L. leichmannii*.

In the test period significant amounts of urinary vitamin B₁₂ activity were detected which were proportional to the amounts given orally. The urinary excretion patterns were similar to those obtained by parenteral injection. Apparently the vitamin is absorbed directly by the nasal mucosa.

Crystalline vitamin B₁₂ crystals produced no irritation of the nasal mucous membranes. In 1 patient numerous nasal polyps and hypertrophy of the mucous membranes apparently did not impede absorption of the vitamin.

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Detroit, Mich.

MICROBIOLOGICAL ASSAYS

- 297 Gregory M. E. The microbiological assay of vitamin B₁₂ in the milk of different animal species. *Brit. J. Nutrition* 8: 340-347, 1954.

Colostrum of cow and goat and the milk of cow, goat, sheep, pig and rat as well as of humans were assayed for vitamin B₁₂ by three different organisms with the following results:

Mean Values From 2 Assays

Species	<i>L. leichmannii</i> assay (m μ g./ml.)	<i>Esch. coli</i> assay (m μ g./ml.)	<i>Ochromonas</i> <i>malhamensis</i> assay (m μ g./ml.)
		Milk	
Cow	29	18	23
Goat	88	11	08
Woman	02	02	02
Sheep	00	00	00
Rat	98	89	87
Pig	100	82	103
		Colostrum	
Cow	19	32	40
Goat	71	88	63

Various methods for preparing the milk samples for assay were compared. The vitamin B₁₂ activity of the milks was due almost entirely to cyanocobalamin. In all milk tested the cyanocobalamin was present in bound form.

- 298 Haenel H. The evaluation of the Euglena test (assay of vitamin B₁₂) with the aid of ultra sound. *Naturwiss.* 41: 143-144, 1954.

The evaluation of the Euglena test by means of turbidimetry was not feasible because the organisms are too large (50

vals up to one week Plasma gave slightly higher counts

In control patients given the smaller dose, measurable activity appeared in most at 4 hours reached a peak at 8 to 12 hours and thereafter slowly declined with significant activity persisting even after a week This absorption curve differed from the early rise observed by others after massive oral doses of vitamin B₁₂, indicating a different mode of absorption It also differed from the rapid disappearance after parenteral administration previously reported The patients with pernicious anemia showed no radioactivity, or only traces, unless intrinsic factor concentrate was given, counts were then within normal range Results were similar when the larger dose was used except that the counts were higher

The above method was compared with a modified Schilling test in 11 control subjects, and in 7 patients with pernicious anemia with and without the use of intrinsic factor In the Schilling test, 1 000 mcg of vitamin B₁₂ was given intramuscularly 24 hours after the oral test dose of 0 46 mcg of Co⁶⁰ vitamin B₁₂ A correlation was found between urinary radioactivity and the plasma or blood activity Duplicate tests on 5 control subjects revealed good reproducibility in the tests, the repeated plasma values correlated a little better than the duplicate urinary excretion percentages

University of Minnesota
Medical School and
Veterans Administration Hospital
Minneapolis Minn

- 294 Rosenblum, C Davis, R L and Chow, H F Comparative absorption of vitamin B₁₂ analogues by normal humans III 5,6-Dichlorobenzimidazole, 5 6-desdimethylbenzimidazole and 5 hydroxybenzimidazole analogues, *Proc Soc Exper Biol & Med* 95 30-32, May 1957

The absorption of vitamin B₁₂ analogues was studied in normal human sub

jects Doses of 1 14 and 2 mcg of labeled, 5,6-dichlorobenzimidazole analogue (DCBA), 5 6-desdimethylbenzimidazole analogue (DMBA) and 5 hydroxybenzimidazole analogue (Bernhauser Factor III) were given Two hours later 1 mg of unlabeled derivative was injected and urinary excretion was measured at 24 hours and in some cases at 48 and 72 hours after daily flushing doses

Slow elimination was not a factor, as one and three-day excretion values did not differ greatly None of the analogues were absorbed to the extent that cyanocobalamin is Desdimethyl and hydroxybenzimidazole were absorbed to only a slight extent the latter being absorbed the least The absorption of the dichlorobenzimidazole analogue was approximately one half that of cyanocobalamin

Clearly, factors other than the cyanogroup affect oral absorption, since all three analogues contain cyanide complexes Of these DCBA with its 2 symmetrically distributed chlorine atoms evinces the greatest absorption tendency One is, accordingly tempted to suggest that the 5,6 disubstitution is a favorable configuration, and that the difference between DCBA and cyanocobalamin lies in preference of the human organism for methyl rather than chloro groups "

Mercek Sharp & Dohme
Research Laboratories
Rahway N J

Johns Hopkins University and
Veterans Administration Hospital
Baltimore Md

- 295 Monto, R W and Howell J T Absorption and excretion of crystal line vitamin B₁₂ when applied to the nasal mucosa *Clin Research Proc* 2 55 56 April 1954

Recent reports on inhalation or nasal instillation of vitamin B₁₂ for pernicious anemia suggest that contact with intrinsic factor is not a prerequisite for the absorption of vitamin B₁₂ through the respiratory mucous membrane In view of these observations it is pertinent to de

termine the vitamin B₁₂ activity in the urine after nasal application

Normal subjects and pernicious anemia patients excrete vitamin B₁₂ in the urine in similar quantities following par-
enteral injection. The urinary vitamin B₁₂ activity following intranasal instillation of the vitamin suggests similar patterns but smaller quantities are excreted than after parenteral administration of equal amounts. A pernicious anemia patient in relapse showed a reticulocytosis of 44.3 per cent when 150 mcg of crystalline vitamin B₁₂ was applied directly to the mucous membrane of the nasal turbinate bone. A single attempt to demonstrate intranasal binding of vitamin B₁₂ with nasal mucus was unsuccessful. Crystalline vitamin B₁₂ in saline solution and as crystals is rapidly absorbed and excreted by the kidneys when applied to the nasal mucosa

Henry Ford Hospital
Detroit Mich

296 Monto R. W. and Howell J. T. Urinary vitamin B₁₂ activity following intranasal administration. *J Lab & Clin. Med* 45 474-477, March 1955

On separate days nasal instillations of 200, 100 and 50 mcg of crystalline vitamin B₁₂ in 0.5 cc saline were given to 2 normal subjects. In addition 2 patients with pernicious anemia in relapse received the nasal mucosa 200 mcg and 150 mcg respectively of vitamin B₁₂ crystals. Urine specimens were collected for a two-hour period before administration and at two, five and eight hours afterward. Assays for vitamin B₁₂ activity utilized *L. leichmannii*

In the test period significant amounts of urinary vitamin B₁₂ activity were detected which were proportional to the amounts given orally. The urinary excretion patterns were similar to those obtained by parenteral injection. Apparently the nasal mucosa is absorbed directly by

Crystalline vitamin B₁₂ crystals produced no irritation of the nasal mucous membranes. In 1 patient numerous nasal polyps and hypertrophy of the mucous membranes apparently did not impede absorption of the vitamin

Henry Ford Hospital
Detroit Mich

MICROBIOLOGICAL ASSAYS

297 Gregory, M. E. The microbiological assay of vitamin B₁₂ in the milk of different animal species. *Brit J Nutrition* 8 340-347, 1954

Colostrum of cow and goat and the milk of cow, goat, sheep, pig and rat, as well as of humans, were assayed for vitamin B₁₂ by three different organisms with the following results

Mean Values From 2 Assays

Species	<i>L. leichmannii</i> assay (m μ g./ml.)	<i>Each coli</i> assay (m μ g./ml.)	<i>Ochromonas</i> <i>malhamensis</i> assay (m μ g./ml.)
Cow	2.9	Milk	2.3
Goat	0.9	1.4	0.8
Woman	0.2	1.1	0.2
Sow	0.0	0.2	0.2
Rat	0.0	0.0	0.0
Ewe	9.6	8.9	8.7
	10.0	8.2	10.3
Cow	3.8	Colostrum	4.8
Goat	7.1	3.2	6.3
		6.2	

Various methods for preparing the milk samples for assay were compared. The vitamin B₁₂ activity of the milks was due almost entirely to cyanocobalamin. In all milk tested the cyanocobalamin was present in bound form.

298 Haenel H. The evaluation of the Euglena test (assay of vitamin B₁₂) with the aid of ultra sound. *Naturwiss* 41 143-144, 1954

The evaluation of the Euglena test by means of turbidimetry was not feasible because the organisms are too large (50

to 60 microns) for this technic. When exposed to ultra sound for one minute the organisms are broken down into particles averaging 3 to 6 microns which brings them into the range of turbidimetric measurements.

Anstalt für Vitaminforschung
Potsdam-Rehbrücke, Germany

- 299 Wolff, R. and Dubost, S. Fixation of vitamin B₁₂ by *Lactobacillus leichmanni* and its application to the determination of bound vitamin B₁₂ in organ extracts. *Biochim Biophys Acta* 14 576-577, Aug 1954

Bound vitamin B₁₂ may be determined in a mixture containing both the bound and free form. Treatment with *L. leichmanni* eliminates the free vitamin.

University of Nancy
Nancy, France

- 300 Bandelin, F. J. and Tuschhoff, J. V. The microbiological determination of vitamin B₁₂ utilizing a mutant strain of *Escherichia coli*. *J. Am. Pharm. A* 43 474-477, Aug 1954

A turbidimetric method for the assay of vitamin B₁₂ utilizing a mutant strain of *Escherichia coli* is described. The growth response curve is reproducible within satisfactory limits. Methionine interferes only when present in amounts 70,000 times that of the vitamin B₁₂. The recovery of cyanocobalamin added to various liver extracts ranges from 96 to 106 per cent. Determinations carried out on a number of injectable liver and desiccated liver extracts indicate that the *E. coli* mutant method gives results which are comparable to those obtained with the official *Lactobacillus leichmanni* method.

It is stated that when methionine interference can be avoided this turbidimetric method may have considerable value because of its simplicity and speed.

Flint-Eaton & Company
Decatur, Ill.

- 301 Grossowicz, N., Aronovitch, J. and Rachmulewitz, M. Determination of vitamin B₁₂ in human serum by a mutant of *Escherichia coli*. *Proc. Soc. Exper. Biol. & Med.* 87 513-514, Dec 1954

The vitamin B₁₂ in human serum was assayed by use of a mutant of *Esch. coli*.

The assay method is accurate and simple, requiring only 40 to 48 hours. The vitamin B₁₂ content of sera of healthy subjects ranged from 200 to 1,000 mcg per cc, in pernicious anemia patients it ranged from 50 to 130 mcg per cc.

Hebrew University
Hadassah Medical School
Jerusalem, Israel

- 302 Ostergaard-Kristensen, H. P. Investigations into the *Euglena gracilis* method for quantitative assay of vitamin B₁₂. *Acta Physiol. Scandinav.* 33 232-237, 1955

Author's summary: Using Ross's modification of Hutner's method for quantitative determination of vitamin B₁₂ with *Euglena gracilis* var. *bacillaris* experiments were carried out which showed that the supernatant from a centrifuged vigorously growing *Euglena* culture contains a thermolabile factor which has a growth-inhibiting effect on *Euglena* cells when they are transferred to a freshly prepared medium containing vitamin B₁₂.

Density and uniformity of growth in the tubes inoculated are increased by washing and diluting the *Euglena* culture used for inoculation. The sensitivity and the accuracy of the method are thereby increased.

University of Copenhagen
Copenhagen, Denmark

- 303 Biological and microbiological methods of estimating vitamin B₁₂. *Nature* 176 384-385, Aug 27, 1955

Summaries of papers were read at a conference on assay of vitamin B₁₂. It was stated that the *L. lactis* assay has largely

been superseded by improved microbiological techniques which employ several organisms. Complicating factors in such assays include the presence of materials closely related to vitamin B₁₂.

Assay with *L. leichmannii* was described and it was stated that probably the best method of measuring serum vitamin B₁₂ levels is to use both this organism and *Euglena gracilis*. *Euglena* provides a highly sensitive method which in serum is specific for vitamin B₁₂.

The problems of using *L. leichmannii* and *Ochromonas malhamensis* for assay of vitamin B₁₂ in animal foodstuffs were also discussed. The *L. leichmannii* assay more closely approximates results by chick assay.

In assay of vitamin B₁₂ in milk *Ochromonas malhamensis*, *Esch. coli* and *L. leichmannii* give closely similar results but the latter is preferred because it provides a simpler method.

- 304 Berman D, Yacowitz H and Weiser H H. A differential microbiological assay for vitamin B₁₂ and pseudo-vitamin B₁₂. *Appl Microbiol* 4 49-52, Jan 1956.

The method employs a differential assay using *L. leichmannii* 7830 and *L. acidophilus* 832 as the test organisms.

Ohio State Univ.
 Columbus, Ohio

- 305 Williams W L, Stiffey V and Jukes T H. Microbiological and chick assay of vitamin B₁₂ activity in feed supplements and other natural products.

Agr. & Food Chem 4 364-367, April 1956.

Recent modifications and improvements of the *Ochromonas* and *Esch. coli* methods as well as the details of a chick growth method for determining vitamin B₁₂ are presented. Certain fermentation materials contain substances which inhibit chick growth so that the chick assay method cannot be used in such cases.

Amer. Iron Cyananilid Company
 Pearl River, N.Y.

- 306 Stapert E M, Goff J W and Stuberfield, L. A vitamin B₁₂ binding substance obtained from *L. leichmannii* 7830. *J. Am. Pharm. A (Sci. Ed.)* 45 309-311, May 1956.

A substance which binds vitamin B₁₂ was obtained from the *L. leichmannii* 7830 organism and the U.S.P. medium in which this organism was cultured. This substance inhibits the growth of *L. leichmannii* 7830.

The Upjohn Company
 Kalamazoo, Mich.

- 307 Robinson F A, Fitzgerald M E H and Grunshaw J J. The microbiological assay of vitamin B₁₂ in liver extracts. I. Experimental design and problems of validity. *J. Pharm. Pharmacol.* 8 635-647, Sept 1956.

Experimental methods are given for the assay of cobalamins in liver extracts using plate and tube assay methods with *B. coli* NCTC 8134 and *L. leichmannii* NCTC 7854 as test organisms.

Allen & Hanbury Ltd.
 London, England

GENERAL

VITAMIN B₁₂ CONTENT, ACTIVITY

- 308 Rosenthal H L and Brown C L, Jr. Vitamin B₁₂ activity of plasma and whole blood from various ani-

mals. *Proc. Soc. Exper. Biol. & Med.* 86 117-120, May 1956.

Authors' summary: Vitamin B₁₂ activity of whole blood and plasma from the human, dog, calf, rabbit, chicken and alligator was studied before and after

alkaline hydrolysis The data show a wide variation of blood and plasma vitamin B₁₂ content between the different species In mammals, the vitamin is almost equally distributed between the erythrocytes and plasma In chicken and alligator, the major portion of the vitamin activity is associated with the nucleated erythrocyte

*Tulane University School of Medicine
New Orleans La*

- 309 Robinson, F A, Fitzgerald, M E H, Fehr, K and Grimshaw, J J Vitamin B₁₂ in crude liver extracts, *Nature* 174 558-559, Sept 18, 1954

Crude liver extracts contain a vitamin B₁₂ binding substance and the vitamin B₁₂ is partly present in bound form, however, binding is not due to the formation of a complex with a protein The conclusion of other investigators that in liver concentrates, cyanocobalamin usually accounts for only a small fraction of total cobalamins is confirmed

*Allen & Hanbury Ltd
London England*

- 310, Hartman A M, Dryden, L P and Riedel G H Vitamin B₁₂ content of milk and milk products as determined by rat assay, *J Nutrition* 59 77-88, May 1956

A vitamin B₁₂ assay method using growth of the depleted normal rat as the criterion of potency has been described and utilized to assay milk and milk products The average vitamin B₁₂ content of 10 lots of raw whole milk was found to be 7.1 mcg per liter Neither pasteurization of milk nor its storage at 0 C for three days had any effect on its vitamin B₁₂ potency

*U S Department of Agriculture
Beltsville Md*

STABILITY STUDIES

- 311 Bartilucci, A and Foss, N E Cyanocobalamin (vitamin B₁₂) I A study of the stability of cyanocobalamin

and ascorbic acid in liquid formulations, *J Am Pharm A (Scient Ed)* 43 159 162, March 1954

Authors summary 'The optimum pH for stability of ascorbic acid in solution was found to be above 6.0 but below 7.0 The optimum pH for cyanocobalamin is between 4.5 and 5.0 The most favorable pH for a mixture of cyanocobalamin and ascorbic acid appears to be between 6.0 and 7.0

'Ascorbic acid was found to be most stable in high concentrations of propylene glycol with distilled water glycerin or Sorbo and a mixture of glycerin and Sorbo Cyanocobalamin was found to be stable in a wide variety of vehicles It has questionable stability in high concentrations of propylene glycol and in vehicles containing glucose A vehicle composed of equal parts of propylene glycol and glycerin afforded maximum stability conditions for a mixture of cyanocobalamin and ascorbic acid There was about 80 per cent retention of cyanocobalamin and 85 per cent retention of ascorbic acid in this vehicle after six months storage at 40 degrees There was no loss of cyanocobalamin and about 10 per cent loss of ascorbic acid on storage for six months at room temperature

'It is suggested that the decomposition products of ascorbic acid play an important part in the decomposition of cyanocobalamin Investigations are continuing on various phases of this problem, and it is expected that the results will be published

*University of Maryland
School of Pharmacy
Baltimore Md*

- 312 Blutz M, Eigen, E and Gunsberg E Vitamin B₁₂ studies—the instability of vitamin B₁₂ in the presence of thiamine and niacinamide *J Am Pharm A (Scient Ed)* 43 651-653 Nov 1954

Authors summary Vitamin B₁₂ is not stable in a B-complex solution at pH

4.25 containing thiamine and niacinamide among its constituents

Oxidation appears not to be a factor in the decomposition of vitamin B₁₂ in the B-complex solution

Large losses of vitamin B₁₂ were found to be a function of the concentration of both thiamine and niacinamide in the solution

- 313 Shenoy, K. G and Ramasarma B Iron as a stabilizer of vitamin B₁₂ activity in liver extracts and the nature of so-called alkali-stable factor Arch Biochem & Biophys 55 293 295 March 1955

The fact that vitamin B₁₂ is more thermostable in Liver Concentrate NF IX than in Liver Fraction 1 NF IX is due to the iron content of the former The vitamin B₁₂ content of Liver Fraction 1 NF IX is destroyed by heating but added vitamin B₁₂ can be protected by addition of FeCl₃

Raptakos Brett & Co
Bombay India

- 314 Macek, T J and Feller, B A A note on stability of the B vitamins in solutions—crystalline vitamin B₁₂ J Am Pharm A. (Scient Ed) 44 254 April 1955

It is possible to prepare aqueous solutions of the B vitamins in which crystalline vitamin B₁₂ is stable for prolonged periods of time at normal storage temperatures

Merck & Co Inc
Rahway N J

- 315 Feller B A and Macek T J Effect of thiamine hydrochloride on the stability of solutions of crystalline vitamin B₁₂ J Am Pharm A (Scient Ed) 44 662-665 Nov 1955

Authors summary: At room temperature crystalline vitamin B₁₂ was found to be stable in aqueous solutions at pH 4 containing 10 mg per cc of thiamine by

hydrochloride alone or in combination with 10 mg per cc of niacinamide Decomposition of crystalline vitamin B₁₂ in aqueous solution was increased appreciably in the presence of thiamine or thiamine decomposition products at elevated temperatures At elevated temperatures the thiazole moiety of the thiamine molecule decomposes crystalline vitamin B₁₂ in aqueous solutions adjusted to pH 4 Data are given to show that crystalline vitamin B₁₂ is stable in B vitamin solutions at pH 3.0 and 4.5 for long periods of time at normal storage temperatures but not when these are heated at 120 degrees

Merck & Co Inc
Rahway N J

- 316 DeMerre L J and Wilson C Photolysis of vitamin B₁₂ J Am Pharm A (Scient Ed) 45 129 134 March 1956

Losses of vitamin B₁₂ caused by exposure to various light sources are as follows: sunlight (8 000 foot-candles) 10 per cent per half hour; artificial light (14 000 foot-candles) about 12 per cent per half hour Effect of ultraviolet light is similar to that of sunlight with monochromatic light photolysis is higher in the short wave range Red light causes no destruction

Medical Laboratories
Army Chemical Center Md

- 317 Garrett, E R. Prediction of stability in pharmaceutical preparations II Vitamin stability in liquid multivitamin preparations J Am Pharm A (Scient Ed) 45 171 178 March 1956

The rates of thermal degradation of ascorbic acid, vitamin B₁₂, folic acid, vitamin A, d-pantothenyl alcohol, and thiamine hydrochloride in a liquid multivitamin preparation have been studied at elevated temperatures and the orders of degradation determined Stability of vitamin components at room temperature was predicted by work performed in a relatively brief time and predictions con

firmed within the estimated error by room temperature shelf stability studies

*The Upjohn Company
Kalamazoo Mich*

- 318 Garrett, E R Prediction of stability in pharmaceutical preparations III Comparison of vitamin stabilities in different multivitamin preparations, *J Am Pharm A (Scient Ed)* 45 470-473, July 1956

The rates of thermal degradation at elevated temperatures and heats of activation of vitamin B₁₂, d pantothenylalcohol and thiamine hydrochloride in a new liquid vitamin preparation have been determined Proof of the validity of a predictive method for room temperature stability is given and possible limitations are discussed

*The Upjohn Company
Kalamazoo Mich*

- 319 Hutchins H H Cravotto, P J and Macek, T J A comparison of the stability of cyanocobalamin and its analogs in ascorbate solution, *J Am Pharm A (Scient Ed)* 45 806-808, Dec 1956

Synopsis The stability of eight vitamin B₁₂ products in 1 per cent ascorbic acid solution in 1 molar acetate buffer at pH 4.0 has been studied during storage at

constant temperatures Cyanocobalamin (crystalline vitamin B₁₂) was shown to be markedly more stable than cyanide free B₁₂ analogs in ascorbate solutions Vitamin B₁₂ concentrates containing mixtures of cyanocobalamin and noncyano analogs were found to be less stable in ascorbate solution than concentrates containing cyanocobalamin exclusively The stability of vitamin B₁₂ concentrates thus appears to be related directly to the concentration of cyanocobalamin present in the concentrate

*Merck Sharp &
Dohme Research Laboratories
Rahway N J*

- 320 Blitz M, Eigen, E and Gunsberg, E Studies relating to the stability of vitamin B₁₂ in B-complex injectable solutions *J Am Pharm A (Scient Ed)* 45 803-806 Dec 1956

Synopsis Room temperature storage studies of various commercial B-complex injectables demonstrate the instability of vitamin B₁₂ in these products Experimental data are presented to show that vitamin B₁₂ is unstable in the presence of thiamine and niacinamide at concentrations from 25 mg to 100 mg per cc of each component in solutions at pH 4.25 but is relatively stable at lower concentrations The mechanism of this decomposition is discussed

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